Global *a priori* identifiability of models of flow-cell optical biosensor experiments

Jason Michael Whyte

ORCID: orcid.org/0000-0002-8575-9504

December 2016

School of Mathematics and Statistics

The University of Melbourne

Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

Abstract

Ideally, a parametric model for a biological system enables prediction of system behaviour for conditions where we lack observations. This necessitates first estimating parameters from some limited data series subject to random error, that is, solving an 'inverse problem'. A solution is some parameter vector that optimizes an objective function. For example, a solution may minimize a sum of squared errors. Multiple (equally valid) solutions may result in unresolvable uncertainty over which is the actual parameter vector.

This is problematic as predictions for a system's observable features — and the unobservable 'state variables' influencing these — may vary drastically with the parameter vector employed. Hence, we cannot confidently use our model to predict system behaviour. Consequently, the effort and resources expended in collecting data provide no benefit.

We may anticipate this problem prior to data collection. We achieve this by testing the combination of a model and proposed experimental conditions for the property of (global *a priori*) identifiability. Testing occurs in an idealised setting which assumes that an infinite, error-free data record is available. It determines those parameter vectors for which model output exactly reproduces such 'idealised data'.

Commonly, errors do not provide information on the model parameters. In this case, it is almost certain that the inverse problem's solution set cannot be smaller than that found by the identifiability test. That is, if the test returns uncountably infinitely-many solutions, we are almost guaranteed of an uninformative study. A test returning a unique solution shows the diametrically opposite outcome; it is at least possible for proposed

experiments to yield a decisive result.

Our interest in identifiability pertains to the modelling of flow-cell optical biosensor experiments. These indirectly monitor the formation and dissociation of complexes of biochemical species. Experimentalists use data with an assumed model for the estimation of parameters representing rate constants.

Often experiments have multiple stages, delineated by an abrupt change in experimental conditions. Accordingly, in certain situations, experimental data is suitably modelled by a type of linear switching system (LSS). As experiments indirectly measure the transfer of mass between forms, and this mass is conserved, suitable models are also 'compartmental'. There is a scant literature on testing LSSs for identifiability, in particular for those which evolve in continuous-time.

Our application leads us to focus on the analysis of continuous-time uncontrolled compartmental LSS of one switching event (ULSS-1). These may suitably model data from a common ('kinetic') type of biosensor experiment having two phases. We propose an appropriate definition for the identifiability of ULSS-1 models and proceed to formulate approaches to testing these for the property. Through use of the symbolic algebra capabilities of Maple, the theory we develop is able to classify each of three test cases. The first two test cases are alternative models for data resulting from the 'simple bimolecular interaction' mechanism. Our results demonstrate the influence of the parameterisation and experimental conditions used in model formulation on the classification obtained. The third — and most complex — test case models data obtained under the 'two-state conformational change' mechanism. Our methods result in the first classification of this model.

The definitive classifications of the test cases demonstrate the viability of our methods for testing ULSS-1 models for global *a priori* identifiability. We give brief consideration to special cases of experiments for which appropriate models are classified more easily. We note future avenues for extension of our methods, including the consideration of experiments having three or more stages.

Declaration

This is to certify that:

i the thesis comprises only my original work towards the degree of Doctor of Philosophy;

ii due acknowledgement has been made in the text to all other material used; and

iii the thesis is fewer than 100 000 words in length, exclusive of tables, bibliographies and appendices.

SIGNED: Jason M. Whyte DATE: December 7th 2016

Dedication

Two people were particularly important to my time spent working on this thesis.

One was my original Ph.D. supervisor, Professor Charles E. M. Pearce of Applied Mathematics at The University of Adelaide. By his diversity of interests and long working hours, Charles was an inspiration.

Charles was much more than a successful academic and prolific author. He cared to ask me about completely un-academic matters such as what I was reading. He enjoyed a pun, and had a wonderful collection of stories from the various phases of his life. I recall warm days sitting in the tea room of the original mathematics building (for a time, the only airconditioned space we had) listening intently to a tale. I recall one about trying to buy points in order to service a Citröen in France without having the appropriate word for the task. Or a story of one-upmanship in finding tighter bounds for an inequality. Or others about time in Japan, including one colourful misunderstanding about obtaining film equipment for underwater striping. On his propensity to tell stories, Charles readily confessed to being in "his anecdotage".

Charles gave me his unwavering support. He was always encouraging me, and would comment to others about the novelty of my project. After recommending me for a position as the Technical Editor of the ANZIAM Journal, Charles imparted knowledge and gave me the opportunity to learn skills that I still find useful today. Charles, with Associate Professor Andrew Metcalfe, was also instrumental in giving me the opportunity to work on an ARC discovery grant with them. I am grateful to them for this further opportunity

to develop skills and broaden my research interests.

Charles was known as someone who gave good advice. I recall two particular gems he gave me. One was to help manage "pussy footing" around with complex tasks. Some red wine would put me in a state where that was not such a problem. He reassured me that most of what mathematicians did was wrong, but just getting something down was the key. It was a "confidence game" he said, quite accurately.

Another useful tip was to not worry excessively about fashions in mathematics. Charles mentioned the book "Big Science, Little Science" on scientific gains coming from outside of the mainstream. I now have some time to read it.

The loss of Charles saddened many, and even now it is not easy to accept. However, we still have the stories.

The second person is my partner, Kathriye Strassnick. Without her I would probably be not much more than a work-unit. She has maintained an unshakeable confidence in me. Kathriye allows me to be something other than a student or research associate. Without her love, this process would have been all too difficult. Together, from Fringe Festivals to foreign lands, we have had great adventures. Together we will find out what the next one will be.

Acknowledgements

In pursuing this Ph.D. I have had the good fortune to meet, learn from, and work with a variety of warm, generous and dedicated people.

The motivation for my thesis arose from time spent in Professor Grant Booker's laboratory in the Department of Biochemistry at The University of Adelaide. Although the lack of definitive results was rather inconvenient at the time, it jolted me into awareness of the central importance of global *a priori* identifiability to inverse problems. Professor Booker and his Ph.D. students Anita Merkel and Sharon Pursglove were always patient with my slow application of lab techniques they taught me, and kind enough to not be upset by the resources I consumed.

Whilst I spent time visiting biochemistry, I lived at The School of Mathematical Sciences at The University of Adelaide. I have a great appreciation and fondness for that community of people. All of the professional staff, in particular Di Parish and Anne Ross, efficiently managed various issues relating to work and conference travel. David Beard, Sue Gray and Dr Paul McCann were always helpful in solving my computing issues or providing advice on how to achieve some end more efficiently.

Resolute, extremely hard-working staff such as Dr Hilary Booth, Dr Tim Svenson, Michael Binkowski, Dr Adrian Koerber, Dr David Parrott and Dr Alison Wolff were kind enough to keep me in mind when allocating computer lab sessions or tutorials. They were just as kind in allowing me to keep away from marking for as long as possible. Working for Geoff Coates in the Mathematics Learning Centre gave me valuable experience in

explaining mathematical concepts to those not terribly interested in mathematics.

I had the benefit of advice, encouragement, or offers of help should I have needed it from various academics over the time of my studies, some of whom also found paid work for me at crucial times. I appreciated such offers from Professor Rey Casse, Associate Professor David Clements, Dr Anna Dostovalova, Dr Janice Gaffney, Dr Peter Gill, Dr David Green, and Professor Patty Solomon. I am especially grateful to Associate Professor Andrew Metcalfe and Professor Tony Roberts from the school, and Professor Lang White from Electrical Engineering for taking the time to read parts of my thesis and provide constructive feedback.

Spending so much time at the School after hours and on weekends was at least partly due to it being such a sociable place. I was fortunate to share an office with Scott Wheeler and interact with his frequent visitors. Due to our school moving buildings, I had the pleasure of sharing three offices with my long-time tea-drinking colleague Nick Crouch. Nick and I spent much time talking about matters such as how we might enthuse Engineering students in first-year mathematics tutorials. (Sometimes this needed something stronger than tea.) I also remember getting tips on tutoring and survival from school stalwarts Jim Andrianopolous, Glenis Crane, and Dr Adrian Vladcoff.

I look back with appreciation at the friendships I formed with fellow postgraduates. I thank Ross McNaughtan for being a partner in scepticism and encouraging me to stop just going to theatre and start reviewing it. This has served me well. When I was "balancing" work and study only through long hours in the office, Aiden Fisher would knock and ask me out for a drink. When I'd say "I'll go out if you come back at 8 p.m.", he would, and I am sure that I was all the better for it. Our conference trip to Vienna will always be memorable.

I always appreciated having a chance to talk to Susana Soto Rojo about non-maths topics, such as what each of us was reading. Similarly, I thank Alex Hanysz for regularly dropping by my office with a coffee invitation to ensure that I got some sunlight, introducing me to Chindogu, and scheduling trips to visit me after I moved to Melbourne. That

he continues to keep contact after some of the not-so-good theatre I've taken him to on these trips is a tribute to his steadfastness. To all of the friends I made as a postgraduate, talking rubbish with you was always entertaining, especially at after-colloquium drinks. Through our shared experiences we came to be reminded that we were much more than our unfinished theses.

My move to Melbourne was made much more pleasant and enjoyable by time spent at MASCOS in Parkville. I enjoyed a wide range of conversations with Dr Andrea Bedini and Dr Owen Dearricott. Kerry Hill was always able to cheerily take care of the various administrative matters relating to my stay. John Baldock was immeasurably important in ironing out technical matters, providing advice, and nursing my ageing laptop towards this completion. I thank Dr Nathan Clisby for his encouragement and for directing some work my way at an important time.

I am particularly appreciative of the efforts my thesis advisors. I thank Professor Peter Taylor for inviting me into his research group, his thorough reading of my thesis draft, and tips on style. I am especially grateful to the MASCOS director Professor Tony Guttmann for his feedback on my work, and kindness in organising a scholarship for me. This freed me from having to do the kind of thankless tasks that sap creativity, giving me the opportunity to resolve some long-standing problems and push this thesis along to its conclusion.

Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 The big picture

A common approach to gaining insight into a physical system is to construct a mathematical model for the system so as to predict the behaviour of its features that are not directly observable. The model is a collection of mathematical expressions relating observable quantities and other variables in the system, and possibly constraints on the values taken by these quantities. The relationships of the model may (or may not) be informed by knowledge, generally incomplete, of the physical processes occurring in the system. A model is an imperfect representation of a physical system, however, to use an enduring quote from the statistician George Box [10] "All models are wrong but some are useful"[1]. The main contribution of this thesis is in anticipating when models of a particular class are not useful, and by complementarity, when models are (at least) potentially useful. Our motivating application is the study of biomolecular interactions.

The mathematical relationships of a model generally feature terms of unknown value called parameters, which, most simply, are constants. Such a collection of relationships may be termed a parametric model. Examples of parameters are:

[1] This notion has appeared in similar forms in other works from Box, such as [11].

(E1) the spring constant in a model of the dynamics of a mass on a spring,

(E2) the fixed resistance and capacitance of a resistor and a capacitor respectively in a model of electrical current for an RC circuit, and

(E3) the rate constant in an expression for the rate of change of the concentration of a chemical species in a chemical reaction occurring in a closed reaction vessel at a constant ambient temperature.

For the sake of simplicity, in this section let us confine our attention to the case in which each parameter represents some constant value. While the use of the term 'model' is widespread, it is possibly open to misinterpretation due to the term having a range of meanings. This thesis will favour an alternative term from the field of systems theory which is unambiguous. Consider a mathematical representation of a physical system in which the values of parameters are not specified. We will call this a **representative system**, which is one that exemplifies a collection of related mathematical systems termed a **model structure**.[2] By using a particular value for the parameter vector in the structure, one obtains a particular mathematical system (or equivalently, model) which belongs to the structure.

Let us formalise this concept by adapting notation from Walter and Pronzato [88].

Definition 1.1. Let M represent a model structure with a parameter vector taking values in a **feasible parameter set** Θ. Then for $\boldsymbol{\theta} \in \Theta$, $M(\boldsymbol{\theta})$ is the system obtained from structure M having parameter vector $\boldsymbol{\theta}$.

Remark 1.1. Any descriptors applied to a structure also apply to its constituent systems, and *vice versa*. Two or more distinct and feasible parameter vectors substituted into a structure may be associated with the same system.

[2] Bellman and Åström [6] used structure to describe a collection of related models in their seminal paper on structural identifiability. In using the term 'representative system' we borrow from set theory where a 'representative element' of a set illustrates common features of members of that set. We will shortly see that the notion of a 'representative system' aids the subsequent discussion of properties of a structure.

In this thesis we have an interest in a particular — and as we will see shortly, very useful — type of property of a structure.

Definition 1.2 (Walter and Pronzato [88])**. A generic property of a structure** M with parameter set Θ is one which holds for almost all $\boldsymbol{\theta} \in \Theta$.

Remark 1.2. The term "for almost all $\boldsymbol{\theta} \in \Theta$" in Definition 1.2 allows for subsets of Θ of zero measure for which the property does not hold. The probability of $\boldsymbol{\theta}$ belonging to such a set is zero, and hence the property holds for the vast majority of feasible parameter values. Such a property is said to hold 'almost everywhere' in the parameter space.

If a structure has a generic property as in Definition 1.2, then the probability is zero that any individual system in the structure will lack the property. Such a property is useful as it allows us to make general statements about the systems in the structure. Shortly we will present generic properties of structures that are of particular relevance to the estimation of a structure's parameters from data.

We assess properties of a structure through consideration of its representative system. To illustrate this, consider structure S_1 composed of systems of a particular class, having parameter set Θ_1. Let $S_1(\boldsymbol{\alpha_1})$ for unspecified $\boldsymbol{\alpha_1} \in \Theta_1$ denote the representative system of S_1. The output of $S_1(\boldsymbol{\alpha_1})$ has particular, defining characteristics. These are algebraic relationships in the parameters which are summarised by some vector, say, $\boldsymbol{\Phi_1}(\boldsymbol{\alpha_1})$. This vector is used to determine whether, in general, a structure has or lacks certain properties. Further, obtaining such a summary from alternative structures enables a comparison of their properties. Figure 1.1.1 schematically illustrates the collection of related systems that comprise a structure, and the defining features of their output.

Suppose we employ a model structure of constant parameters to represent a particular physical system. In this case we typically assume that there is one true value for each parameter. This assumption is informed by the principle of parsimony.

As mathematical systems are the building blocks of structures, let us consider some

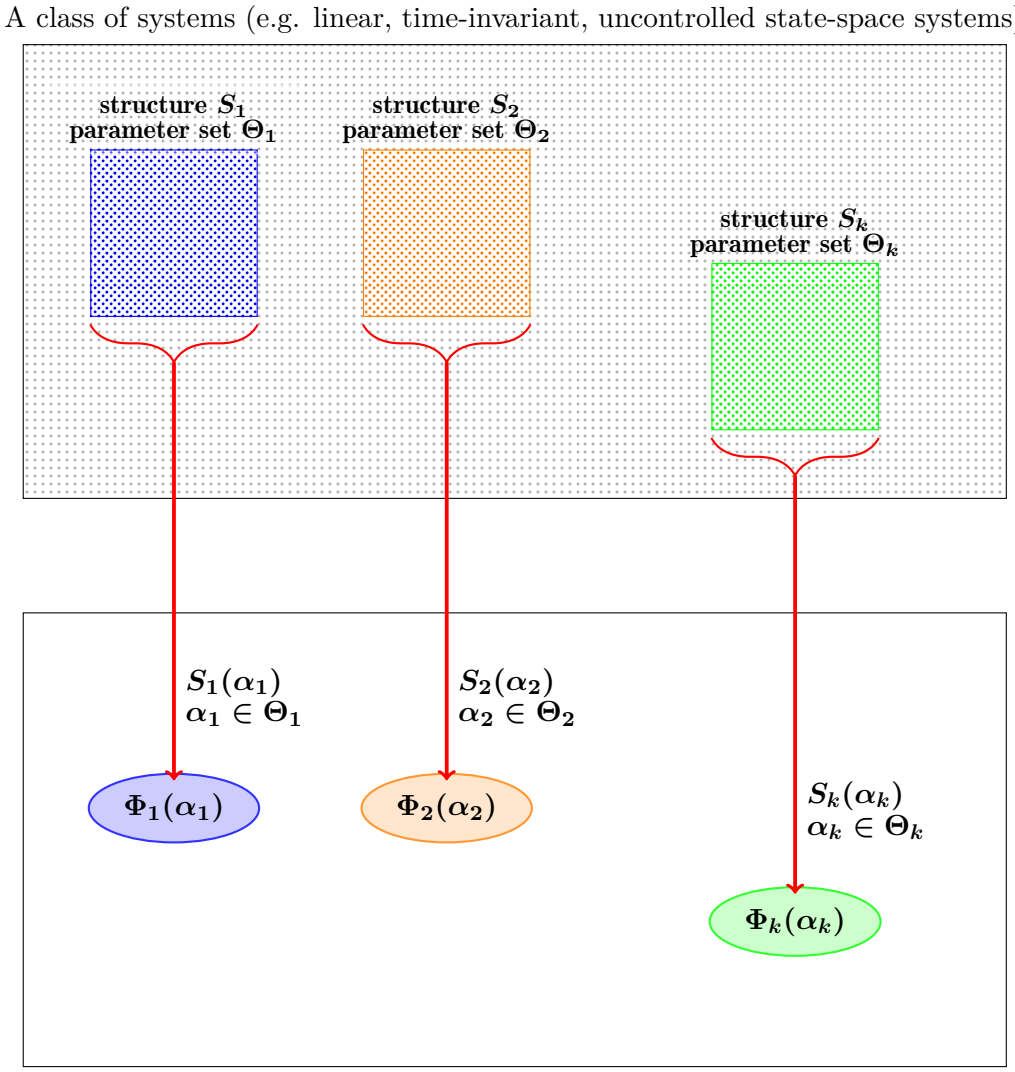


Figure 1.1.1: The top rectangle is a 'relationship space' containing all of the mathematical systems of a given class. Colours indicate collections of related models comprising structures (shown by squares). The lower rectangle is a 'descriptive space'; a structure is summarised by its representative system. This has certain defining features which enable scrutiny of the structure's properties.

particularly useful classes of systems. One flexible class is the **state-space systems**. Structures of these have found use in modelling a variety of physical systems, including those drawn from mechanical, electrical, and chemical applications, such as examples (E1), (E2), and (E3) above. (For further examples, see [46, 48].)

A state-space system has two essential features. The first is a system of first-order ordinary differential equations (ODEs) which describe the rates of change of certain **state variables** over time. State variables represent features of the physical system which we may not be able to measure. Examples include the velocity of the mass in (E1), the current at a point in the circuit in (E2), and the concentration of a particular chemical species in (E3).

The second feature is the presence of one or more functions of the state variables, modelling the observable features of the physical system. Each of these quantities is an **output** of the state-space system. To take one example, suppose in (E3) it is not possible to measure the concentration of a species directly. However, by passing light of a particular wavelength and known intensity through the reaction vessel and measuring the amount absorbed, the Beer–Lambert law allows an estimate of the concentration. In this case, absorbance is modelled by an output that is a function of the species concentration, which itself is modelled by a state variable.

A **controlled state-space system** is used to represent a physical system that is subject to at least one influence (or control) which arises externally to the system. Such an external control may be termed an **input**. Examples of inputs are an applied mechanical forcing term in (E1), or the applied voltage in (E2). In controlled state-space systems, inputs appear explicitly in some part of the system. Otherwise, such as in (E3) where there are no inflows of mass once the reaction vessel is sealed, no input is applied. Such a physical system is appropriately modelled by an **uncontrolled state-space system**.

To illustrate important features of structures of state-space systems, let us consider a simple example drawn from the study of the movement of tracers (chemical elements or radioactive isotopes) in the human body. Batschelet *et al.* [5] considered experiments

aiming to quantify the movement of lead in the human body over time. The body absorbs lead from the biosphere through air, water and dietary intake. Experiments added the stable lead isotope ^{204}Pb to the diet of human subjects. It is known that tracers are transported between the skeleton, the blood, and the tissues. The mathematical model structure used to describe the distribution of ^{204}Pb in an individual treated each of these regions as a **compartment**, and together these comprise the **system**. Anything outside of the system is termed the **environment**.

A schematic description of the hypothesized movement of tracers in a subject is given by Figure 1.1.2. This informed the model structure used to model the rates of tracer transport between compartments. Each square represents one of the bodily compartments known to receive lead. It is assumed that the contents of each compartment are well-mixed, that is, that the concentration of tracer is the same at all points in the compartment. Each of the structure's state variables correspond to the concentration of tracer in one particular compartment. When a concentration is directly measurable, the corresponding state variable is also an output of the model structure. Thin arrows show the direction of tracer transport from one compartment to either another compartment or to the environment through bodily excretion. Each arrow has an associated parameter that incorporates features of the transport process, such as a diffusion constant. The uptake of tracer by the subject from the environment (an input) is shown by the thick arrow directed into the blood compartment.

The structure associated with Figure 1.1.2 provides an example of when features of constituent models are subject to constraints. As concentrations and lead uptake are non-negative quantities, the state variables (and hence outputs) and inputs of any suitable model structure must also be non-negative. As matter is not created or destroyed in the experiment, any suitable structure must enforce this through ensuring the movement of tracer satisfies conservation of mass conditions. These conditions impose constraints on the structure's parameters. Under such restrictions on states, outputs, inputs and parameters, an appropriate model structure for the physical system is composed of systems belonging

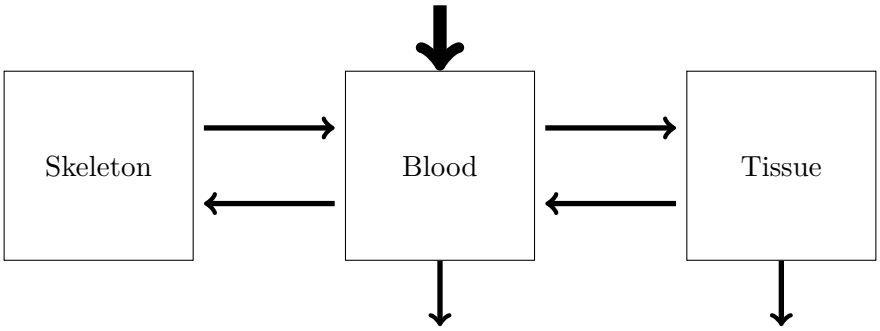


Figure 1.1.2: A compartmental model for the transport of radioactive tracers in a human subject. (From Seber and Wild [73], a simplified form of Figure 1 in Batscheler *et al.* [5].)

to the class of **compartmental models**. Structures of compartmental state-space models have found use in modelling a variety of biological systems, including the spread of an infectious disease through a population (Vynnycky and White [87]) and the change in membrane voltage over time in neurons (Sterratt *et al.* [76]).

A detailed classification of state-space systems requires further terminology. For example, terms such as 'linear' or 'nonlinear' indicate how the output of a system depends on inputs. The term 'time-invariant' indicates a system for which relationships between state variables, inputs and outputs are fixed.[3] If the state variables of a system are described by a difference equation it is a **discrete-time system**. A system for which state variables are modelled by a set of differential equations is a **continuous-time** system. We make particular use of the class of continuous-time, linear, and time-invariant compartmental state-space models in this thesis.[4]

The credible use of a model structure in prediction of system behaviour is contingent on the structure's ability to adequately approximate the system. Judging this ability typically requires us to first solve the problem of determining feasible parameter values

[3]These terms are formally defined in Section 3.4.

[4]This class of models is presented in Section 3.6.

for which predictions made by the structure 'best' agree with system observations[5]. This 'inverse problem' may be ill-posed, that is, have multiple solutions. This is troublesome when the alternative parameter vectors lead to substantially different predictions of the behaviour of unobservable features of the system. Thus, one cannot use the model structure to confidently predict the behaviour of the physical system as a whole.

Another consequence of multiple solutions is that one cannot answer "What is the value of each parameter?" Constraints on parameters suggested by the physical system may allow either the deduction of each parameter's true value, or at least restrict the range of plausible values to an acceptable range. However, if an inverse problem has uncountably infinitely-many solutions, discrimination between alternatives is impossible. Thus there is always the possibility that the study of a physical system will be less informative than hoped, or at worst, an uninformative use of time and resources.

There are various concrete examples of where it is not possible to distinguish between alternative solutions to an inverse problem. One arises in experimental design relating to a factorial experiment. Let us consider an experiment where some physical process is subject to three explanatory variables — say A, B, and C — which influence some observable quantity Y. If each variable takes either a low value (represented as -1) or a high value (+1), this is termed a full 2^3 factorial experiment. The combinations of variable values are shown in Table 1.1.1.

Suppose that N observations of Y (Y_i for $i = 1, \ldots, N$) are recorded under conditions as given in the rows of Table 1.1.1. Each set of experimental conditions occurs with equal frequency. A typical first step in modelling the experimental values of Y is to assume a multiple linear regression model structure relating Y to the experimental variables, say

[5] A model structure is an imperfect representation of a physical system, and observations of the system are typically subject to random errors, the nature of which may not be known. As a result, it is very unlikely that there exists some parameter value for which the output of a deterministic model structure exactly reproduces real data. The judgement of the 'best' parameter value(s) may depend on which 'objective function' is chosen to quantify the agreement of observations and the corresponding model-derived predictions.

A	B	C	ABC
-1	-1	-1	−
-1	-1	+1	+
-1	+1	-1	+
-1	+1	+1	−
+1	-1	-1	+
+1	-1	+1	−
+1	+1	-1	−
+1	+1	+1	+

Table 1.1.1: The set of possibilities for variables A, B, and C, and the accompanying sign of ABC, in a full 2^3 factorial experiment.

the values of A and B, and BC defined as the interaction of B and C. The representative model has the form:

$$Y_i = \beta_0 + \beta_1 \mathrm{A}_i + \beta_2 \mathrm{B}_i + \beta_3 (\mathrm{BC})_i + \epsilon_i \,, \quad i = 1, \ldots, N, \tag{1.1.1}$$

where parameters β_j, $j = 0, \ldots, 3$ are to be estimated from the data, and ϵ_i represents an random error term. We assume that these errors are suitably modelled by some random process.

Assumption 1.1. In the absence of prior information, we make certain assumptions about errors, some of which depend on the nature of the structure employed. For a 'linear regression model' as in (1.1.1), these are the Gauss–Markov assumptions (see, for example, Devore [23, Chapter 12]). These include the assumptions that all errors are uncorrelated, and are drawn from the same probability distribution which has mean zero and some fixed, finite variance. That is, that the errors do not provide any information on the parameters.

We may also assume these conditions for structures in which outputs are nonlinear in the parameters, although this context requires variations to some of the other Gauss–Markov assumptions (see, for example, Greene [31, Chapter 7]).

Under the assumptions described, we may use the sum of squared errors (SSE) as our objective function in an inverse problem, where an error is the difference between an observation and its corresponding model prediction. Those parameter values which minimize the SSE are our parameter estimates.

When we assume a structure that is linear in the parameters, minimizing the SSE with errors subject to the Gauss–Markov assumptions is termed a ordinary least squares (OLS) problem. When an assumed structure is nonlinear in the parameters and assumptions on the errors such as those of Greene [31] apply, minimizing the SSE is termed a nonlinear least squares (NLS) problem.

Often it is not feasible to collect data for all possibilities in a full factorial experiment due to the time or expense required. An alternative is to perform a fractional factorial experiment which uses only some of the variable combinations. Consider an experiment which collects only those $2^{3-1} = 4$ combinations in Table 1.1.1 where ABC=+1. Hence we can show

$$\mathrm{ABC} = +1: \quad \mathrm{A(ABC)} = \mathrm{A}^2\mathrm{BC} = \mathrm{A}. \tag{1.1.2}$$

As $\mathrm{A}^2 = 1$ for $\mathrm{A} = \pm 1$, $\mathrm{A}^2\mathrm{BC} = \mathrm{A}$ in (1.1.2) suggests that $\mathrm{BC} = \mathrm{A}$. This relationship is seen in Table 1.1.1 by inspection of rows 2, 3, 5 and 8 in which ABC=+1. Hence, the values of A and BC are not independent.

This result shows that for the planned fractional factorial experiment, it is not possible to distinguish between the effects of A and BC on Y. This prompts the rewriting of (1.1.1) as

$$Y_i = \beta_0 + (\beta_1 + \beta_3)\,\mathrm{A}_i + \beta_2 \mathrm{B}_i + E_i\,, \quad i = 1, \ldots, N, \tag{1.1.3}$$

for E_i an error term.

Equation (1.1.3) shows that it is possible to estimate $(\beta_1 + \beta_3)$ from the fractional factorial experiment data, but not β_1 and β_3 individually. Hence, the use of (1.1.3) in

an inverse problem would lead to a properly implemented parameter estimation process returning multiple values of β_1 and β_3.

The fractional factorial experiment above is a particular example of a general situation where parameter estimates are not unique. Consider the archetypal matrix equation form of a linear regression equation such as (1.1.1) for $i = 1, \ldots, N$. It is assumed that the vector of N observations $\mathbf{y}$, a vector of P parameters $\boldsymbol{\beta}$, and the matrix of explanatory variables $\mathbf{X}$ of size $N \times P$ are related through

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon}, \tag{1.1.4}$$

where $\boldsymbol{\epsilon}$ is a vector of errors that are subject to the Gauss–Markov assumptions (recall Assumption 1.1). In this case, it is appropriate to attempt to obtain parameter estimates by solving an OLS problem.

By the standard formula, the least-squares estimate(s) of $\boldsymbol{\beta}$ (denoted $\hat{\boldsymbol{\beta}}$) are the solutions of

$$\left(\mathbf{X}^\top \mathbf{X}\right) \hat{\boldsymbol{\beta}} = \mathbf{X}^\top \mathbf{y}. \tag{1.1.5}$$

Equation (1.1.5) provides a unique $\hat{\boldsymbol{\beta}}$ only if $\mathbf{X}$ is of full rank. Otherwise, there are either infinitely many solutions for $\hat{\boldsymbol{\beta}}$, or none. To illustrate the former case, consider the earlier factorial experiment where ABC=+1 and each set of conditions is only used once. Recasting (1.1.1) in terms of (1.1.4) gives

$$\mathbf{y} = \begin{bmatrix} 1 & \text{-1} & \text{-1} & \text{-1} \\ 1 & \text{-1} & 1 & \text{-1} \\ 1 & 1 & \text{-1} & 1 \\ 1 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} + \boldsymbol{\epsilon}, \tag{1.1.6}$$

where we note that columns two and four of $\mathbf{X}$ are identical. As this $\mathbf{X}$ is rank-deficient, infinitely many $\hat{\boldsymbol{\beta}}$ follow as solutions to the OLS problem.

In general, we cannot inspect a model structure and so readily ascertain that estimates of its parameters will be non-unique or otherwise. However, for certain classes of

structures, we can anticipate the likely result of parameter estimation. This is determined by testing the model structure[6] for the property of global *a priori* identifiability.

To explain this further, it is necessary to sharpen the description of inverse problems given earlier. Suppose the observations of some physical system of interest are collected over time. The inverse problem is defined by these observations — a record of sampled, noisy data of limited duration – and a model structure which is an imperfect representation of the physical system. In testing a structure for global *a priori* identifiability, rather than solving the inverse problem, one considers a related, idealised problem. In the simplest case (say, for an uncontrolled structure) one assumes that an infinite, error-free observation record is available and that the structure is indeed the correct representation of the physical system. The test of a structure for global *a priori* identifiability determines those distinct feasible parameter vectors for which the structure precisely reproduces the idealised output. The solution set of this idealised inverse problem is determined exactly.

Let us suppose that we can model real data as the sum of predictions from our structure and some process which generates random errors. In considering a state-space structure we may generalise Equation (1.1.4) and write the observation as t, $\mathbf{y}_t$, as

$$\mathbf{y}_t = \mathbf{f}(\mathbf{x}_t, \mathbf{u}_t, \boldsymbol{\theta}) + \boldsymbol{\epsilon}_t \,, \tag{1.1.7}$$

where $\mathbf{f}(\mathbf{x}_t, \mathbf{u}_t, \boldsymbol{\theta})$ is the structure's prediction given $\mathbf{x}_t$ and $\mathbf{u}_t$ representing the state vector and input at time t respectively, and $\boldsymbol{\theta}$ representing the parameter vector.

Suppose that the errors in (1.1.7) do not give any information on the parameters $\boldsymbol{\theta}$, as in Assumption 1.1.[7] In such a case, the set of solutions to a real inverse problem (say, those obtained by minimizing a SSE) cannot have fewer elements than the set of solutions

[6] and, most generally, the experimental design which determines the conditions (such as applied inputs) under which observations of the system are made.

[7] We note that from the beginnings of structural identifiability, as in Bellman and Åström [6], conditions on the errors are not made explicit. This indicates an implicit supposition that conditions as in Assumption 1.1 hold. This is reasonable in the absence of any information on the errors.

to the associated idealised inverse problem. Under such assumptions on the errors, when a test anticipates non-uniqueness of parameter estimates, it is unlikely that we will obtain a unique estimate for each parameter from the real data produced by the intended study.

In order to justify this statement, let us consider some aspects of parameter estimation using real data. In this thesis we have particular interest in prediction functions such as $\mathbf{f}$ in Equation (1.1.7) that are a sum of exponentials. These arise from certain classes of structures we will employ later. There is a large literature on the difficulties of obtaining accurate or unique parameter estimates from sums of exponentials employed in an inverse problem when data is sampled or subject to error. For example, Berman [7] presented an overview of numerical issues in the fitting of models to data. These included non-uniqueness of parameter estimates in 'ill-conditioned' inverse problems, including those which employ sums of exponentials.

Varah [84] further explored the consequences of ill-conditioned inverse problems. Using simulated data subject to truncation of significant digits, the paper presented diagrams to show regions of a SSE surface where a wide range of parameter values can produce SSE values close to its global minimum. Varah [84] also used simulated data subject to error to demonstrate an 'uncertainty region' around a least squares estimate of parameters. Within such a region, a continuum of parameter values gave the same SSE, which prevented the inverse problem considered from having an unique solution.[8]

The impediments to obtaining unique parameter estimates described above demonstrate a limitation to what we can discover by testing a structure for global *a priori* identifiability. As long as the errors conform to Assumption 1.1, it is reasonable to expect that the test will give a lower bound on the number of solutions to an actual inverse problem. That is, the test may underestimate the number of solutions of the inverse problem. Hence, the acceptable result of a test is not sufficient to ensure a satisfactory resolution of an inverse problem in a given study. However, as this test does not require the collection

[8]Further consideration of the solutions to an inverse problem using noisy data requires a discussion of global *a posteriori* identifiability of a structure, which is beyond the scope of this thesis.

of data, we can use the test result to anticipate and reconsider a study likely to be futile. This allows us to redirect time and resources to a study showing greater promise.

While in certain fields it is standard practice to test a proposed model structure for global *a priori* identifiability, this is not the case in others. Consider, for example:

> We contend that this [testing a model structure for global *a priori* identifiability] is, as of yet, an under-appreciated issue in biological modelling and more particularly, cell biology. (Roper *et al.* [70])

However, there are some particularly instructive papers where non-uniqueness of parameter values estimated from data is emphasized and used to improve or critique a biochemical model. For example, Schmidt *et al.* [72] fitted a structure representing a biochemical system to data, and noted those parameters for which there were multiple estimates. Elimination of these parameters produced a structure for which parameters may be uniquely determined from data. Cox *et al.* [18] considered a model of cells progressing through various stages towards becoming tumour cells. Their numerical study simulated counts of cancer cells for when a subject was exposed to some carcinogen for a period of time. It was demonstrated that different parameter values fit this simulated data equally well. However, these alternatives predicted very different cell counts for when the conditions were changed to expose a subject to a different level of carcinogen. By considering the non-uniqueness of parameter estimates obtained from a numerical record, the approaches of Schmidt *et al.* [72] and Cox *et al.* [18] are a less general — and less powerful — approach to evaluating the well-posedness of the inverse problem than testing a model structure for *a priori* global identifiability.

The particular inverse problems of interest to this thesis relate to studies of the interactions of biomolecules. Studies of the formation and breakdown of complexes of biomolecules can contribute information to the study of biochemical pathways. In molecular biology laboratories, the study of interacting species commonly uses a flow-cell optical biosensor. These report a response in real time that is influenced by the amount of complex

present in a monitored reaction volume[9]. A structure is assumed to represent the rates of binding and unbinding processes believed to occur in an experiment. The structure contains parameters such as rate constants that are estimated from experimental data[10].

One use of optical biosensors is in providing information for the modelling of complex biological systems, such as biochemical networks. The associated model structures tend to have many state variables and parameters. Consider a comment on the difficulties of defining and using these, made in the context of modelling genetic regulatory networks:

> The principal modelling challenges come from incomplete knowledge of the biochemical reactions underpinning most networks, and the dearth of quantitative data for kinetic parameters required for detailed mathematical models. (Casey *et al.* [15, Page 27])

Such a lack of quantitative information could be resolved by scrutinizing individual interactions from the network with optical biosensor experiments, and using the resulting data to estimate rate constants.

It is common for flow-cell optical biosensor experiments to have two distinct stages or **phases**, caused by a change of experimental conditions. In considering these, Whyte [91] showed that structures of linear switching systems[11] are suitable for representing some proposed interactions of chemical species. Some examples of the time course of response produced by a biosensor for such experiments are shown in Figure 1.1.3. Experimental response of this type has informed this thesis. Note the abrupt change in behaviour of the output curves that is suggestive of switching behaviour. This indicates the change from one phase of an experiment to the next.

The flexibility of the switching system structure gives them an advantage over other classes of system:

> ... some industrial systems, such as those with abrupt changes in their dynam-

[9] The operation of optical biosensors is explained more fully in Chapter 2.

[10] Some elementary definitions from chemistry are given shortly in Section 2.2.

[11] These are defined in Section 3.7.

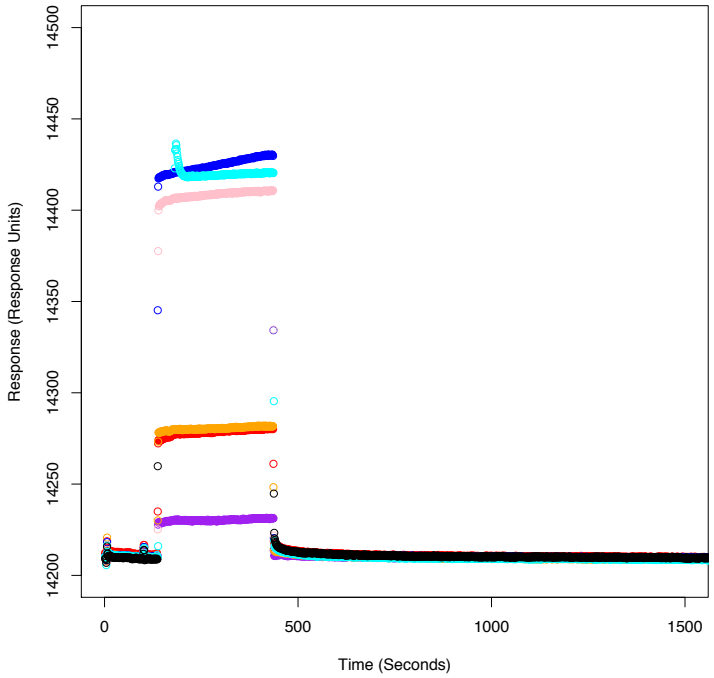


Figure 1.1.3: Response curves for a series of flow-cell optical biosensor experiments where each colour represents the data obtained for a particular concentration of reactants.

> ics, can not be appropriately described by the famous linear time-invariant state-space representation. Such systems can be adequately described by the class of stochastic switching systems called piecewise deterministic systems or jump systems. (Boukas [9])

In describing linear switching systems (within the class of linear hybrid systems) Vidal *et al.* [85] claim that "many real processes can be approximated arbitrarily well by models in this class." This ability has led to the use of switching systems in fields such as economics, control of mechanical systems, the automotive industry, and air traffic control[12].

The structures of linear switching systems (LSS) used to model chemical interactions

[12]For references, see, for example, [27, 78].

in this thesis are termed "compartmental". This is because they incorporate the law of conservation of mass that interactions are subject to. Investigation of the properties of compartmental LSS requires a different approach from that used for other types of LSS.

Although the term 'linear switching system' was not used, structures of such systems have found use in modelling biological systems, some time before their prominence in control theory. For example, a well-known text on compartmental analysis published in 1983 considers LSS briefly, referring to them as time-varying systems where

> ... some (or all) of the rate constants suddenly change from one steady value to another. This is a particular feature of many pharmacokinetics trials, and the change may be caused by exercise by a subject previously at rest or the intake of food by a subject who had previously been fasting. (Godfrey [29, Page 215]).

The structure of compartmental LSS presented in Godfrey [29] was not tested for global *a priori* identifiability and no references are given on the matter. This is notable given that Chapters 5 to 7, and sections in Chapters 8 to 10 of the book consider global *a priori* identifiability for a variety of model structures. It appears that applications of the type described above did not stimulate the development of mathematical theory for testing LSS structures for global *a priori* identifiability. This is shown by the state of the literature; until quite recently the theory considered only structures having fixed algebraic relationships between outputs, states, and inputs. The lack of exploration of global *a priori* identifiability for LSS structures may have been a result of the desired use of the mathematical models employed. For example, a LSS structure may be of interest (at least initially) for its ability to reproduce observations, rather than for its ability to predict the value of unobservable state variables.

The analysis of LSS structures poses challenges that are not faced in the analysis of linear or nonlinear structures that are time-invariant. We have had the benefit of access to the symbolic algebra capabilities of Maple^{TM} [50], which we use extensively in derivations and calculations. The usefulness of such software in the time after Godfrey's book is made clear by the comment in Raksanyi *et al.* [64] that solving the equations central to *a priori*

identifiability problems by hand was "... tedious, hazardous, or even impossible", and the title of a paper by Zheng [105] entitled "Computer algebra is indispensable in some problems of mathematical biology".

Since the time of Godfrey [29], analysis of compartmental structures has entered the realm of systems theory. Recent research in this area has broadened the range of model structures tested for global *a priori* identifiability by considering certain classes of LSS structures. Discrete-time structures received much of this attention. Some of the published methods assumed that the time of a switching event was unknown. This is an unnecessary complication for models of flow-cell optical biosensor experiments where the switching time is fixed by the experimentalist. At times the structures considered were subject to conditions which are not justifiable for the structures considered in this thesis. Further, many of the studies use a definition of 'identifiability' which is not equivalent to that of global *a priori* identifiability considered here.[13]

The optical biosensor experiments of primary interest to this thesis are of two phases. This motivates the development of a theory of global *a priori* identifiability suitable for structures of continuous-time, compartmental uncontrolled linear switching systems of one switching event.

The body of this thesis is organized as follows. Chapter 2 begins with an introduction to flow-cell optical biosensors and an overview of certain concepts from chemistry. These assist the discussion of chemical interactions studied through biosensor experiments to occur later. An inspection of the optical biosensor literature shows that the mathematical models for chemical interactions and the resultant biosensor response occur in a variety of forms. Further, the mathematical models may have implicit assumptions. To ensure a clear understanding of what is included or implicit in interaction models, we give an overview of one type of flow-cell optical biosensor experiment of two phases and the composition of the biosensor response.

Chapter 3 provides an introduction to relevant aspects of systems theory from the

[13] A review of this literature is given in Section 1.4.

literature. This leads to a review of the property of global *a priori* identifiability. This background is used in Chapter 4 to propose a definition of global *a priori* identifiability for structures of continuous-time uncontrolled linear switching systems of one switching event (ULSS-1), and tests of such structures for the property.

Chapter 5 applies the theory outlined in Chapter 4 to test cases drawn from the optical biosensor literature. This allows us to ascertain the utility of the proposed tests. The test cases are representations of the 'simple bimolecular model' (formalised in Whyte [99]) and the 'two-state conformational change model' (Whyte [97]). The proposed approaches are able to make a judgement on all test cases. This success has led to the first published classifications of structures representing optical biosensor data. Ultimately, our methods demonstrate the importance of the parameterisation used in specifying a model structure.

Various models of biomolecular interactions occurring on a flow-cell optical biosensor are yet to be tested for global *a priori* identifiability. The methods presented here provide a foundation for further classifications. With this in mind, closing remarks and notes on possible future studies in areas related to the subject of this thesis are made in Chapter 6.

Finally, appendices are given showing the Maple^{TM} commands and output. These show the application of the global *a priori* identifiability tests to the test cases, and some simulations investigating properties of model structures. Also given is an appendix on some terminology from chemistry for completeness.

We progress in the next section to give an illustration of what it means for a structure to be globally *a priori* identifiable. Following the introduction to chemical systems in Chapter 2 and properties of structures in Chapter 3, we will present a more detailed view of the property in Section 3.6.4.1.

1.2 An introduction to the property of global *a priori* identifiability of a structure

Consider a structure M with feasible parameter set Θ. Suppose we obtain a continuous-time output from the structure's representative system $M(\boldsymbol{\theta})$ ($\boldsymbol{\theta} \in \Theta$), say $\mathbf{y}_{\boldsymbol{\theta}}$, that is error-free and infinite in extent. We refer to output of this type as 'idealised'. Similarly, consider system $M(\boldsymbol{\theta}')$ ($\boldsymbol{\theta}' \in \Theta$) from which idealised output $\mathbf{y}_{\boldsymbol{\theta}'}$ is obtained.

Structure M is termed globally *a priori* identifiable (see, for example, Audoly *et al.* [3]) if for almost all values of $\boldsymbol{\theta} \in \Theta$ (as in Remark 1.2), the condition $\mathbf{y}_{\boldsymbol{\theta}'} = \mathbf{y}_{\boldsymbol{\theta}}$ is satisfied only if $\boldsymbol{\theta}' = \boldsymbol{\theta}$.

Let us demonstrate this structure classification, and some alternatives, with an illustration. We are assisted in this regard by introducing some elementary notation. We will present further notation to enable our mathematical arguments in Section 3.1.1.

Note 1.1. The set of natural numbers is denoted by $\mathbb{N} = \{1, 2, \ldots\}$. The field of real numbers is denoted by $\mathbb{R}$. The subset of $\mathbb{R}$ containing only positive values is denoted by $\mathbb{R}_+$. For $n \in \mathbb{N}$, the n-dimensional Euclidean space is denoted by $\mathbb{R}^n$. An element of $\mathbb{R}^n$ is an n-tuple of real numbers.

For $a \in \mathbb{R}_+$, the Heaviside step function $\mathcal{H}_a : \bar{\mathbb{R}}_+ \to \{0, 1\}$ is defined by

$$\mathcal{H}_a(t) = \begin{cases} 0, & 0 \leq t < a, \\ 1, & t \geq a. \end{cases} \tag{1.2.8}$$

Suppose that $M(\boldsymbol{\theta})$ has $\boldsymbol{\theta} = (\theta_1, \theta_2, \theta_3)^\top \in \mathbb{R}^3_+$. Further suppose that with $\boldsymbol{\theta} = \boldsymbol{\theta}^*$ (a particular parameter vector) system $M(\boldsymbol{\theta}^*)$ has output $\mathbf{y}_{\boldsymbol{\theta}^*}$. Consider the positive orthants of $\mathbb{R}^3$ shown in the three subfigures of Figure 1.2.4. Each shows a green circle indicating $\boldsymbol{\theta}^*$, and any other feasible parameter values $\boldsymbol{\theta}'$ such that $y_{\boldsymbol{\theta}'} = y_{\boldsymbol{\theta}^*}$.

Figure 1.2.4a has only the feasible parameter value $\boldsymbol{\theta}' = \boldsymbol{\theta}^*$. Figure 1.2.4b has some

separation between the multiple feasible $\boldsymbol{\theta}'$. Figure 1.2.4c, shows a continuum of $\boldsymbol{\theta}'$. If the first, second, or third outcome occurs for almost all $\boldsymbol{\theta}^* \in \mathbb{R}^3_+$, then M is globally *a priori* identifiable, locally *a priori* identifiable, or *a priori* unidentifiable, respectively.

If a model structure is the correct representation of a physical system, one may think of $\boldsymbol{\theta}^*$ as representing the 'true' value of a parameter vector. This true value is not known prior to the collection of data and subsequent solution of an inverse problem. As such, we cannot anticipate exactly which properties the particular model for the system will have. However, by considering generic properties of a proposed model structure, one may at least ascertain which properties are very likely to hold for any model in the structure.

This thesis encounters each of the three structure classifications above. The simplest case concerns the structure proposed in Whyte *et al.* [100] for a chemical reaction system with rates of change of the concentrations of species X, Y, R and S given by

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix}\mathrm{X}\\ \mathrm{Y}\\ \mathrm{R}\\ \mathrm{S}\end{bmatrix}(t) = \begin{bmatrix}-k_3 & 0 & 0 & 0\\ 0 & -k_4 & 0 & 0\\ k_3 & 0 & -k_7 & 0\\ 0 & k_4 & 0 & -k_8\end{bmatrix}\begin{bmatrix}\mathrm{X}\\ \mathrm{Y}\\ \mathrm{R}\\ \mathrm{S}\end{bmatrix}(t), \qquad \begin{bmatrix}\mathrm{X}\\ \mathrm{Y}\\ \mathrm{R}\\ \mathrm{S}\end{bmatrix}\Bigg|_{t=0} = \begin{bmatrix}X_0\\ Y_0\\ 0\\ 0\end{bmatrix},$$

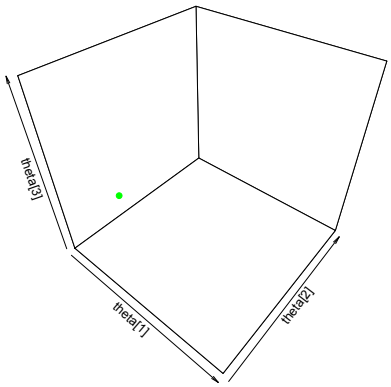
and outputs

$$\mathbf{y}(t) = \begin{bmatrix}0 & 0 & 1 & 0\\ 0 & 0 & 0 & 1\end{bmatrix}\begin{bmatrix}\mathrm{X}\\ \mathrm{Y}\\ \mathrm{R}\\ \mathrm{S}\end{bmatrix}(t).$$

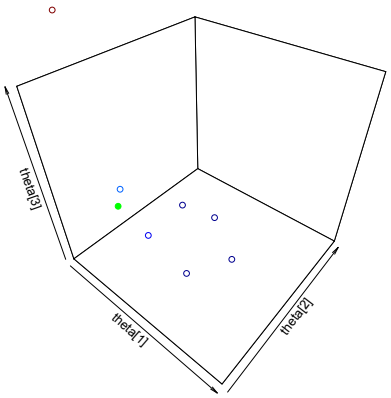
The structure is shown to be locally *a priori* identifiable in Section 3.6.4.1.

The other classifications are seen for structures of linear switching systems modelling chemical interactions in flow-cell optical biosensor experiments. These structures are inspected in Chapter 5.

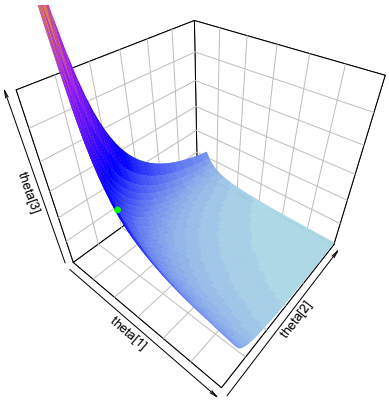
In order to give some introduction to these structures, we present two here. The structure representing the three-parameter form of the "simple bimolecular interaction" has



(a) A unique $\boldsymbol{\theta}'$ satisfies $y_{\boldsymbol{\theta}'} = y_{\boldsymbol{\theta}^*}$.



(b) Multiple – but separated – $\boldsymbol{\theta}'$ satisfy $y_{\boldsymbol{\theta}'} = y_{\boldsymbol{\theta}^*}$.



(c) A continuum of $\boldsymbol{\theta}'$ satisfy $y_{\boldsymbol{\theta}'} = y_{\boldsymbol{\theta}^*}$.

Figure 1.2.4: The points or surface shown represent alternatives $\boldsymbol{\theta}'$ to the parameter vector $\boldsymbol{\theta}^*$ (green circle) for which $y_{\boldsymbol{\theta}'} = y_{\boldsymbol{\theta}^*}$. If, for almost any value of $\boldsymbol{\theta}^*$, the alternatives to $\boldsymbol{\theta}^*$ are as in (a), (b), or (c), then the structure is termed *a priori* globally identifiable, *a priori* locally identifiable, or *a priori* unidentifiable, respectively.

representative system relating biosensor output $\mathbf{y}$ to state variables representing species concentrations $\mathbf{x}$:

$$\begin{aligned} \dot{\mathbf{x}}(t,\boldsymbol{\theta}) &= \mathbf{A}_{\gamma(t)}(\boldsymbol{\theta}_{\gamma(t)})\mathbf{x}(t,\boldsymbol{\theta}), \quad \mathbf{x}(0,\boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}_1), \\ y(t,\boldsymbol{\theta}) &= \mathbf{C}_{\gamma(t)}(\boldsymbol{\theta}_{\gamma(t)})\mathbf{x}(t,\boldsymbol{\theta}). \end{aligned} \tag{1.2.9}$$

The switching function $\gamma(\cdot)$ is defined by

$$\gamma(t) = \begin{cases} 1, & 0 \leq t < t_1\,, \\ 2, & t \geq t_1\,. \end{cases} \tag{1.2.10}$$

The structure's representative system has particular features:

$$\begin{gathered} \boldsymbol{\theta_1} = (k_a, k_d, \beta_1)^\top \in \mathbb{R}^3_+, \quad \boldsymbol{\theta_2} = (k_d)^\top \in \mathbb{R}^1_+, \\ \boldsymbol{\theta} = \boldsymbol{\theta}_1 \in \Theta = \mathbb{R}^3_+, \quad \alpha_1 > 0, \\ \mathbf{A_1}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 & k_d \\ k_a\alpha_1 & -k_d \end{bmatrix}, \quad \mathbf{A_2}(\boldsymbol{\theta_2}) = \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix}, \\ \mathbf{C_1} = \mathbf{C_2} = \begin{bmatrix} 0 & 1 \end{bmatrix}, \qquad \mathbf{x_0}(\boldsymbol{\theta_1}) = \begin{bmatrix} \beta_1 & 0 \end{bmatrix}^\top. \end{gathered} \tag{1.2.11}$$

We show that the structure defined by (1.2.9), (1.2.10) and (1.2.11) is globally *a priori* identifiable in Section 5.2.

We observe a less favourable result for a closely-related alternative structure. This is the four-parameter form of the "simple bimolecular interaction", from which the three-parameter form is derived. The four-parameter structure has representative system of the form given by (1.2.9) and (1.2.10) as before, with specific features given by

$$\begin{gathered} \boldsymbol{\theta_1} = (k_a, k_d, B_0, \rho_A)^\top \in \mathbb{R}^4_+, \quad \boldsymbol{\theta_2} = (k_d)^\top \in \mathbb{R}^1_+, \\ \boldsymbol{\theta} = \boldsymbol{\theta}_1 \in \Theta = \mathbb{R}^4_+, \quad \alpha_1 > 0, \\ \mathbf{A_1}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 & k_d \\ k_a\alpha_1 & -k_d \end{bmatrix}, \quad \mathbf{A_2}(\boldsymbol{\theta_2}) = \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix}, \\ \mathbf{C_1} = \mathbf{C_2} = \begin{bmatrix} 0 & \rho_A \end{bmatrix}, \qquad \mathbf{x_0}(\boldsymbol{\theta_1}) = \begin{bmatrix} B_0 & 0 \end{bmatrix}^\top. \end{gathered} \tag{1.2.12}$$

In Section 5.3 we will see that this four-parameter structure is *a priori* unidentifiable. This result indicates that scrutiny of a proposed model structure can ascertain if it is fit for its intended purpose. Such scrutiny may also indicate how to reformulate a structure such that the result has desirable properties lacking in its progenitor.

In the following section we will review the literature on testing structures of linear switching systems for global *a priori* identifiability. We will formally meet continuous-time uncontrolled linear switching systems in Section 3.7.

1.3 Some remarks on various notions of 'identifiability'

The literature has a variety of usages of 'identifiability', some of which do not closely relate to global *a priori* identifiability of a structure as we define it.[14] At least some of this variation has resulted from the broad range of disciplines interested in inverse problems (Jacquez [36]). To avoid possible misunderstanding, we briefly consider the meaning of some alternative terms here. This section serves as an introduction to the usages of 'identifiability' that arise in the literature on linear switching systems, which we will discuss in Section 1.4.

A term such as 'identifiability' may appear by itself (Jacquez and Grief [37]), or be preceded by a descriptor such as 'deterministic' (Godfrey [29]) or 'structural' (Bellman and Åström [6]) rather than *'a priori'* (Audoly *et al.* [3]).

It appears that this diversity of terminology often relates to whether a 'model', 'system' or 'structure' is being inspected to ascertain the presence of some property. These objects are not always equivalent. In order to discuss the literature, it is necessary to understand the common use of 'model' as meaning a particular structure, rather than an individual element of a structure having a particular parameter vector. The following explains one perceived misuse of terminology:

[14] We present the concept formally for an uncontrolled structure in Definition 3.17.

> The identifiability problem is concerned with the theoretical existence of unique solutions and so is strictly a mathematical and *a priori* problem. Note that it refers to specific experiments on a specific model, so it is properly *model identifiability* for the given experiments and not system identifiability. Unfortunately, the term system identifiability is used widely in the literature and really confuses the issue. (Jacquez and Grief [37, Page 201])

The distinction drawn by Jacquez and Grief [37] relates to the hypothetical conditions under which idealised data is obtained from a controlled model, such as, which set of inputs is applied to the model to elicit output. A test for 'system identifiability' assumes that output is available for a set of inputs — say the set of piecewise continuous functions — such that one is able to deduce the invariants of the model (structure), as we introduced in Figure 1.1.1.[15] In 'model identifiability' however, one considers the output resulting from some restricted set of inputs, possibly only one input, and we may not be able to deduce invariants from this output. Thus, in general, the notions of 'system identifiability' and 'model identifiability' are not equivalent.[16] This distinction is the same as that drawn between structural and deterministic identifiability, as mentioned briefly in Whyte [91].

[15] We will formally define invariants in Equation (3.5.16).

[16] We will see in Chapter 3 that for the uncontrolled structures we consider, global *a priori* identifiability in terms of structure output as presented in Definition 3.17 is equivalent to the concept as expressed in Definition 3.19 in terms of invariants. There is no difference between 'model identifiability' and 'structure identifiability' in this setting. This is not necessarily the case for controlled structures. Certain texts begin an exploration of identifiability by using controlled structures subject to a particular input, and then proceed to illustrate global *a priori* identifiability as if all possible inputs were available. (See, for example Seber & Wild [73, Chapter 8].) We suspect that similar omissions of subtleties has played some role in the confusion of system and model identifiability.

We can readily extend global *a priori* identifiability as presented in Definition 3.17 to apply to controlled structures by including the inputs applied to the structure and collating the corresponding outputs. A test using Definition 3.17 would then serve as a test of the controlled structure for 'model identifiability', but possibly not 'system identifiability'. If the list of inputs employed does not allow us to determine the structure's invariants, then we have insufficient information to test the structure for system identifiability. This demonstrates that, in general, Definition 3.17 is not equivalent to Definition 3.19 for all structures.

In this thesis we consider uncontrolled structures, for which the distinction between model identifiability and system identifiability dissolves. Thus, the use of '*a priori* identifiability of an uncontrolled structure' is unambiguous, with '*a priori*' emphasising the application of the testing process to the structure prior to the collection of data.

There is another point on which descriptions of identifiability in an *a priori* sense for continuous-time structures may differ. Testing such a structure for global *a priori* identifiability is often described as occurring in an idealized framework. Within this, it is assumed that the structure is the correct representation of the physical system, and that an infinite record of error-free data is available. At other times, it may be assumed that such idealized data is available for some time interval $[0, \tau]$ $(\tau > 0)$. These alternative conditions are equivalent for the structures considered in this thesis; having response on an interval is equivalent to having response for all time in $\bar{\mathbb{R}}_+$ by the theory of analytic continuations.

Certain usages of 'identifiability' in the literature relate to properties of structures that differ substantially from those variants described above. These may consider the set of solutions of an actual — rather than idealised — inverse problem. The membership of such a set is used to classify a structure in terms of (global or local) *a posteriori* identifiability (also known as numerical identifiability, see Jacquez and Greif [37] or Godfrey [29, Chapter 8]). We make this classification in a manner analogous to that used to classify a structure based on solutions of an idealised inverse problem.[17]

Certain authors from outside of the disciplines of mathematics or systems theory have explored *a posteriori* identifiability of a structure (sometimes without using this term) by determining the solutions to an inverse problem when 'data' is obtained by simulating outputs from the structure given a particular parameter value. In such a case, the property is certainly not generic (as in Definition 1.2), and hence is a much less general property than global *a priori* identifiability of a structure.

[17] We will describe this process in Definition 3.17.

1.4 An overview of the literature on linear switching system structures and global *a priori* identifiability

To the author's knowledge, there are very few publications relevant to the study of global *a priori* identifiability of structures of linear switching systems (also known as jump linear systems, and by permutations of these terms) in the literature. This is particularly the case for continuous-time structures. This section gives a brief review of some recent papers. These are presented in order by year of publication.

The earliest potentially relevant offering is the 2002 paper by Vidal *et al.* [85] entitled "Observability and Identifiability of Jump Linear Systems". The paper considered discrete-time uncontrolled LSS. In particular, states $\mathbf{x}$ and outputs $\mathbf{y}$ were related by

$$\begin{aligned} \mathbf{x}_{t+1} &= \mathbf{A}(\lambda_t)\mathbf{x}_t + \mathbf{v}_t\,, \\ \mathbf{y}_t &= \mathbf{C}(\lambda_t)\mathbf{x}_t + \mathbf{w}_t \end{aligned} \tag{1.4.13}$$

with discrete state at time t given by λ_t and additive noise given by $\mathbf{v}_t$ and $\mathbf{w}_t$. It was assumed that switching times were unknown, needing to be estimated from the data. Conditions were given on the time between switching events and on the system parameters of the subsystems under which it was possible to determine the switching times uniquely. For the ULSS-1 systems we consider in this thesis there is no need to estimate switching times; the one switching time is considered known — as it would be in an experiment generating actual data — and is taken as an arbitrary non-negative real number. The observability part of [85] was concerned with conditions and a process that would allow determination of the sequence of discrete and continuous states. This information is not of particular interest to a test of a ULSS-1 structure for global *a priori* identifiability in itself.

Vidal *et al.* [85] described their notion of identifiability in their introduction: "Another important issue is whether the model itself can be inferred from data, *i.e.*, whether it is identifiable." ([85, Page 3614]). The paper considered this in Section 3.2 where it was assumed that a data vector was available which would allow the calculation of one pair of

system matrices for each of the subsystems that produce the output, that is, the $\mathbf{A}(\lambda_t)$ and $\mathbf{C}(\lambda_t)$ in (1.4.13) that occurred for all values taken by λ. Conditions were then given which defined the set of system matrices obtainable from those calculated which generate the same output as the observation vector.

From this we can see that the authors' notion of identifiability of a structure is unlike that of global *a priori* identifiability used in this thesis.[18] First, [85] required data to perform calculations (hence the test is for a numerical or *a posteriori* property) whereas testing a structure for global *a priori* identifiability does not. Second, the study of [85] did not assume a particular form for the system matrices of the original system, it used whichever system matrices that were returned by the identification algorithm applied to data.

These features make [85] substantially different from a true consideration of identifiability. The effect of testing a structure M for global *a priori* identifiability is to return all systems that are both output equivalent to the structure's representative system $M(\boldsymbol{\theta})$ and which have system matrices of the same pattern as their corresponding matrix in $M(\boldsymbol{\theta})$. The omission of this second requirement in Vidal *et al.* [85] means that the process employed is actually finding the set of systems that are indistinguishable[19] from the particular system they originally determined from data.

Overall, the process given in Vidal *et al.* [85] is not immediately useful for the matter of this thesis. However, there may be some merit in further considering the section on realizability. This section provides conditions under which discrete-time structures are indistinguishable. Suppose that it is possible to adapt the conditions given so that they are appropriate for continuous-time structures. Restricting the models considered to only those from the structure of interest will form the basis for a test of a model structure for global *a priori* identifiability. Any adaptation of the approach will require the recon-

[18] We employ a definition consistent with that used by various authors, based on that of Denis-Vidal and Joly-Blanchard [22]. We present this in Definition 3.17.

[19] We present generic indistinguishability in Section 3.5.4.1 of this thesis. Vidal *et al.* [85] consider a non-generic form of indistinguishability.

ciliation of the theorem given in Vidal *et al.* [85] with seemingly inconsistent conditions used elsewhere in the paper. As a result, we postpone any further evaluation for future consideration.

Note 1.2. No comment was made on whether the approach proposed was suited to linear switching systems that were composed of compartmental or positive subsystems.

Whyte [90, 91] considered global *a priori* identifiability as it pertained to continuous-time ULSS-1 structures.[20] The papers were motivated by the study of biomolecular interactions observed with flow-cell optical biosensor experiments considered in this thesis. They provided the first considerations of structure properties for this application. As mass is conserved in biomolecular interactions, testing methods proposed were designed to be appropriate for a ULSS-1 structure of systems composed of compartmental linear time-invariant subsystems.[21]

The approach of [90, 91] considered invariants present in the output of the ULSS-1 structure.[22] As a LSS is piecewise LTI, techniques from the analysis of LTI structures provided inspiration.[23] Most of the approaches used to test a LTI structure for global *a priori* identifiability require the structure to be generically minimal.[24] This property is often said to be a consequence of the generic properties of controllability (or reachability) and observability[25]

Whilst such definitions are appropriate for LTI structures, they may not hold for

[20] These papers informed the definition employed in this thesis.

[21] We will meet linear time-invariant (LTI) structures in Section 3.6 and the compartmental type of these in Definition 3.24.

[22] We will explain some subtleties of this in Definition 3.19.

[23] We will give an overview of some methods of testing a LTI structure for global *a priori* identifiability in Section 3.6.4.

[24] This property is defined in Section 3.6.3.4.

[25] These properties are defined in Sections 3.6.3.1–3.6.3.3. We note that there is some variation in the terminology used to define notions of 'minimality'; see, for example, Kalman [39] and van den Hof [82].

compartmental LTI structures.[26] However, a method using the Laplace transform does not require a structure to have the generic properties required by other methods.[27] Hence, we adapted this approach in [90, 91] to propose a test of a ULSS-1 structure for global *a priori* identifiability. This test is suited to structures for which it is not necessary to estimate switching times, as we consider in this thesis. We may apply the test without modification to a ULSS-1 structure of systems which have states and outputs that take real values.

Vo Tan, Millérioux and Daafouz contributed a conference paper,"Invertibility, flatness and identifiability of switched linear dynamical systems: an application to secure communications" [61], to the literature in 2008. This is quite similar to a 2010 journal follow-up by these authors, "Left invertibility, flatness and identifiability of switched linear dynamical systems: a framework for cryptographic applications" [62]. As such, we will discuss the papers together.

The papers [61, 62] considered properties of discrete-time, single-input single-output (SISO) linear switching systems where states, inputs and outputs take real values. Note 1.2 also applies to these two papers. The cryptographic application considered meant that any possible switching sequence was permitted, unlike the applications considered in this thesis where the switching sequence is known. The papers describe 'identifiability' for their systems by "...a parameter of a discrete-time dynamical system is *identifiable* if it can be rewritten as a unique function of the input, the output and their iterates." (See [62, Page 149].)

Note 1.3. The definition of 'identifiability' given differs from that we employ (given in Section 3.5.3) as it implied that the property holds throughout the parameter space, not allowing for sets of measure zero where the property does not hold.

Note 1.4. The papers appear to describe global identifiability of a parameter, without mak-

[26] We will explain this in Section 3.5.2.

[27] This is the 'Laplace Transfer approach', which we will meet in Section 3.6.4.

ing any reference to local identifiability. A structure judged as locally *a priori* identifiable may be useful in certain circumstances.

Two parameter estimation methods were employed in testing a LSS for 'identifiability'; one for when the switching sequence was known and a second for when the switching sequence was not known. Both required that the output of the system was 'flat'.[28] The first identification method assumed that parameter estimation could occur because "... the usual persistently exciting (PE) conditions ..." apply ([62, Page 148]). These conditions were also required in the second parameter estimation routine. The PE conditions were not defined and references were not given to illustrate the conditions. Hence, it is unclear whether PE conditions apply in general.

Vo Tan *et al.* [61] considered an example to illustrate their proposed methods. This example had inconsistencies between the number of discrete states present and the enumeration of the total number of switching sequences on a particular interval of time. Specifically, the LSS considered had four discrete states and yet the total number of switching sequences over a period of two consecutive time points was given as four, rather than the 16 expected. These issues were resolved when the example was reconsidered in [62] for a LSS of only two discrete states. However, there were other inconsistencies over the interpretation of parameters and their assumed values, although the latter may be merely typographical errors.

The application of the second parameter identification method to the example was very brief in both [61] and [62]. In each case the method resulted in conditions that related some functions of the parameters to constants. We expect that the process of testing a structure for global *a priori* identifiability will lead to purely parametric relationships. The authors' example showed that given a particular value for each parameter of a LSS, the authors' conditions may be used to deduce whether the parameter estimates are unique.

[28] From [62, Definition 2.4,Page 147], "A flat output of a dynamical system is an output variable y_k such that all system variables can be expressed as a function of y_k and a finite number of its forward/backward iterates."

This is not as useful as concluding that a condition holds for almost all feasible values of the parameters. We conclude that the approach outlined in [61] and [62] is not actually a test of a LSS for global *a priori* identifiability as we define it in Section 3.5.3.

Adapting the second approach such that it does test a LSS for global *a priori* identifiability will require calculations with symbolic expressions rather than with numerical values. In such a case, it is reasonable to expect that the computations required in [61,62] will be more complex and time consuming. Results of these computations may also be more difficult to interpret than in the straightforward example given in those papers. We may consider suitable modifications to the techniques of Vo Tan *et al.* in a later study. However, it is notable that the method assumed that the time(s) of switching between subsystems was unknown. This makes the approach unnecessarily complicated for the linear switching systems we consider in this thesis for which the switching time does not require estimation as it is taken as arbitrary but fixed.

The authors claimed on Page 149 of [62]; "The unicity of the solution of an identification procedure is directly related to the notion of parametric identifiability (Nõmm and Moog 2004; Anstett, Bloch, Milléroux, and Denis-Vidal, 2008)." It is appropriate to relate this statement to the specific notion of global *a priori* identifiability of a structure considered in this thesis. A test of a structure for global *a priori* identifiability occurs under idealized conditions. For the purposes of illustration, let us suppose that observations are represented by the predictions made by some model in our assumed structure subject to additive random error. Further suppose that we intend to estimate parameters by minimizing a residual sum of squared errors. We follow the literature in assuming that knowledge of the errors does not provide any information on the parameters of the structure.

In such a case, determining that the structure is globally *a priori* identifiable means that it is possible for a properly implemented parameter estimation using real data to return a single value for each parameter, but this is not guaranteed. Thus, one may say that global *a priori* identifiability of a structure is necessary — but not sufficient — for a

properly implemented parameter estimation to return a unique value for each parameter.

The 2010 conference paper of Petreczky *et al.* [60] "Identifiability of Discrete-Time Linear Switched Systems" showed that the authors had a notion of 'identifiability' that is similar to some of the papers cited above. As such, this interpretation is not equivalent to that used in this thesis and elsewhere.

To illustrate this, consider the outline of the concept:

> "More precisely, a parametrized system structure is a map from a certain parameter space to a set of dynamic systems. Such a parametrized model structure is said to be (structurally) identifiable, if no two different parameter vectors yield two models whose input-output behaviour is the same. If there are two parameter vectors such that the corresponding models have the same input-output behaviour, then no amount of measurements will be enough to determine which of the two parameter vectors is the true one."[29] ([60, Page 141])

The authors presented a more mathematical definition in Theorem 6 of [60].

The definition of 'identifiability' given in [60] is not indistinguishability (as presented by [85]), but it is not quite structural identifiability (or global *a priori* identifiability as preferred in this thesis). The points made in Note 1.3 on the use of the term 'structural' apply to this paper, not just for identifiability, but also for the definition of structural minimality given in Definition 12 on Page 144 of [60]. Further, Note 1.4 also applies to this paper.

As for [61, 62, 85], Petreczky *et al.* [60] confined their study to discrete-time LSS due to "... their simplicity and and their relevance for the system identification community." The paper considered LSS for which the states, inputs and outputs take real values, and the observation of Note 1.2 applies here.

[29] In this quote, we note the use of 'model' to describe both a 'structure' and a particular system obtained from the structure for a particular parameter vector, adding to the diversity of uses of the two terms seen in the literature, and potential confusion over terminology. We will describe this diversity further in Section 3.5.3.

On Pages 141-142 of Petreczky *et al.* [60], in describing 'identifiability' for hybrid systems, the authors stated "To the best of our knowledge, only [33,28] address the concept of identifiability." The papers cited are Vo Tan *et al.* [61] and Vidal *et al.* [85] respectively, which we discussed earlier in this section. Petreczky *et al.* noted that unlike [61], their approach to testing a LSS for identifiability did not require the property of flatness, which was also required by [62].

Page 142 of Petreczky *et al.* [60] emphasized "Notice here we view *the switching signal as an external input and any switching signal is admissible.*" This assumption played a key role in the approach of the paper. The authors required that any LSS must be structurally minimal (as they define it) and this was a consequence of the LSS having the properties of span reachability and observability. To test the LSS for these properties, calculations assumed that all possible switching sequences of length less than or equal to the number of state variables were possible.

This assumption is not reasonable in all settings. For example, chemical interactions occurring in an optical biosensor experiment as we consider in this thesis must have the association phase occur before the dissociation phase. This experimental setup determines the subsystem of the LSS which is used to model the experiment at any particular time. Hence, the LSS models the system with a known switching sequence, and it is not sensible to suggest that other sequences can apply. From this example, it is possible that the tests of the LSS for span reachability and observability cannot proceed as [60] intended. This suggests that the approach of [60] is not appropriate for all applications.

The requirement that a LSS structure must have the property of 'structural minimality' before it can be tested for identifiability was explained on Page 145 of [60] where it was stated that the LSS "...can be completely determined by the minimal component of the switched system." The authors continued: "However, let us remark that one can also think of cases, when finding the minimal subsystems of the parametrization explicitly is not straightforward." The approach of Whyte [90, 91] may have an advantage in this case as it does not require an explicit representation of the minimal part of a ULSS-1 in order

to test it for global *a priori* identifiability.

Section 3.4 of Petreczky *et al.* [60] considered some examples of LSS to demonstrate that the properties of the individual subsystems of the LSS did not necessarily determine LSS properties. The examples presented did not have dependency between the unknown elements of the subsystem matrices, unlike compartmental systems. Hence Note 1.2 applies again.

Section 4 of Petreczky *et al.* [60] added a new element to the literature by considering "semi-algebraic parameterizations". Their approach to testing a structure that was a semi-algebraic parametrization drew on technical definitions from logic and real algebraic geometry. The lack of an application of their method to an example obscured how it may be applied in practice.

In the description of checking a semi-algebraic parametrization of a LSS, the authors' interest was in generating structures that were indistinguishable from a given structure (as noted in the discussion of Vidal *et al.* [85] above) rather than testing a structure for global *a priori* identifiability. The authors gave a logical formula for deciding whether a LSS is identifiable or not identifiable (as they define it).

Presenting this formula requires first reproducing some of the preliminary material given in Petreczky *et al.* [60]. Consider a discrete-time LSS Σ represented by

$$\begin{aligned} x(t+1) = A_{q(t)}x(t) + B_{q(t)}u(t) \quad \text{and} \quad x(0) = x_0\,, \\ y(t) = C_{q(t)}x(t), \end{aligned} \tag{1.4.14}$$

where $x(t)$, $u(t)$, and $y(t)$ represent the continuous state, input, and output at time $t \in T$ respectively. Further, $q(t) \in Q$ is the discrete state at time t for Q a finite set of discrete states. For each $q \in Q$, the matrices of the LSS in (1.4.14) are $A_q \in \mathbb{R}^{n\times n}$, $B_q \in \mathbb{R}^{n\times m}$, and $C_q \in \mathbb{R}^{p\times n}$. The notation $(n, Q, \{(A_q, B_q, C_q) | \, q \in Q\}, x_0)$ serves as a short-hand representation of (1.4.14).

Consider system Σ_1 of the form of (1.4.14), and a possibly distinct system of the

same number of state variables,

$$\Sigma_2 = \left(n^a, Q, \left\{\left(A_q^a, B_q^a, C_q^a\right) \middle| \, q \in Q\right\}, x_0^a\right) \quad \text{with} \quad n^a = n.$$

Also, for matrix $S \in \mathbb{R}^{n\times n}$, the proposition $IsRank_n(S)$ is true if S has rank n, and is false otherwise. Then S is a LSS isomorphism $S : \Sigma_1 \to \Sigma_2$ if and only if

$$IS(\Sigma_1, \Sigma_2, S) = \\ (Sx_0 = x_0^a) \wedge \left(\bigwedge_{q\in Q} \left(SA_q = A_q^a S\right) \wedge \left(SB_q = B_q^a\right) \wedge \left(C_q = C_q^a S\right)\right) \wedge IsRank_n(S) \tag{1.4.15}$$

is true.[30]

Petreczky *et al.* [60] drew on the preliminaries above to present their semi-algebraic formula:

$$\begin{aligned} \Phi_{ident} = \forall \theta_1, \theta_2 \in \mathbb{R}^d : \Big((\theta_1 \in \Theta) \wedge (\theta_2 \in \Theta) \wedge \theta_1 \neq \theta_2\Big) \to \\ \neg\Big(\exists S \in \mathbb{R}^{n\times n} : IS\left(\Sigma(\theta_1), \Sigma(\theta_2), S\right)\Big). \end{aligned} \tag{1.4.16}$$

Let us consider the meaning of the proposition Φ_{ident} in deciding whether alternative parameter vectors in the structure Σ can produce the same output. Consider parameter vectors θ_1 and θ_2 from some set Θ that are not equal. In (1.4.16), this implies that there does not exist some real $n \times n$ matrix S that can act as a LSS isomorphism as in (1.4.15) between the systems $\Sigma(\theta_1)$ and $\Sigma(\theta_2)$. This implication holds for all feasible θ_1 and θ_2. That is, if Φ_{ident} is true, then there does not exist any $\theta_2 \neq \theta_1$ for which the output of $\Sigma(\theta_2)$ is the same as that of $\Sigma(\theta_1)$.

The truth or falsehood of Φ_{ident} was used by Petreczky *et al.* [60] in deciding whether or not a generically minimal LSS structure Π is identifiable according to a lemma.

Lemma 3, Petreczky *et al.* [60]. Let Π be a structurally minimal semi-algebraic parametrization. Then Π is structurally identifiable if and only if Φ_{ident} is true over $\mathbb{R}$.

[30] We note that the conditions placed on the matrices of Σ_1, Σ_2 and S by (1.4.15) extend those which constitute a similarity transform applied to the matrices of the representative system of a LTI structure. See, for example, van den Hof [82].

Equation (1.4.16) is notable as expressing the property through a formula is uncommon in the literature; as far as we are aware, Whyte [90, 91, 94] provided the only other examples. The advantage of the formula as expressed in [90, 91] (with a refinement made in Whyte [94]) is that it can also discern when a structure is locally *a priori* identifiable. Further, applying the associated test returns any relationships between parameters that cause a LSS structure to lack the property of globally *a priori* identifiability. We can use this information to direct the reparameterisation of an *a priori* unidentifiable structure such that we obtain a more useful one.[31]

Petreczky *et al.* [60] concluded with a statement "Moreover, identifiability of all the linear subsystems is obviously a sufficient condition for identifiability of a linear switched system." ([60, Page 149]). The statement is correct, but again expects that the LTI structures defined by the LSS structure can be considered individually. It is not clear how one would do this for the continuous-time ULSS-1 structures we consider in this thesis. In modelling a flow-cell optical biosensor experiment, the first LTI structure represents the association phase, which runs for some non-zero length of time before the switch to the dissociation phase. The initial state of the dissociation phase structure depends on the parameters of the association phase structure and the switching time. In general, there is no simple expression for the initial state of the dissociation phase structure. Lacking this detail, the structure is not fully specified. As such, it is not appropriate to test the dissociation structure for global *a priori* identifiability with standard methods.

The method of Whyte [94] was designed specifically to apply to continuous-time ULSS-1 structures representing flow-cell optical biosensor interaction models. The method sought to overcome a potential impediment to the further use of Whyte [90,91] in classifying these structures. In particular, it was expected that (in general) the previous methods would become more difficult to apply to a structure as the number of state variables increased beyond two. Rather than testing a ULSS-1 structure for global *a priori* identifiability directly, the new approach chose to assemble conditions which might be sufficient

[31] We will see an example of this in Chapter 5.

to allow the inference that a structure was globally *a priori* identifiable. In order to test the new method, Whyte [94] analysed a more complex test case than that considered previously. The classification of this "two-state conformational change model" (having three state variables) as globally *a priori* identifiable was only the second published classification of a flow-cell optical biosensor model.[32]

We may wonder if the literature on testing a time-varying linear structure for global *a priori* identifiability can contribute to our problem. One description of a continuous-time, uncontrolled linear time-varying system is

$$\begin{aligned} \dot{\mathbf{x}}(t,\boldsymbol{\theta}(t)) &= \mathbf{A}(t,\boldsymbol{\theta}(t))\mathbf{x}(t,\boldsymbol{\theta}(t)), \quad \mathbf{x}(0,\boldsymbol{\theta}(t)) = \mathbf{x_0}(\boldsymbol{\theta}(0)), \\ y(t,\boldsymbol{\theta}(t)) &= \mathbf{C}(t,\boldsymbol{\theta}(t))\mathbf{x}(t,\boldsymbol{\theta}(t)), \end{aligned} \tag{1.4.17}$$

having state vector $\mathbf{x} \in \mathbb{R}^n$, output $\mathbf{y} \in \mathbb{R}^m$, matrices $\mathbf{A}$ and $\mathbf{C}$ have appropriate dimensions, and $\boldsymbol{\theta}(\cdot)$ is a time-varying parameter vector.

A general technique for the testing of time-varying linear structures was proposed in Audoly *et al.* [3]. Let us consider the application of this technique to the type of linear switching system (a ULSS-1) described by (1.2.9) with (1.2.10).

The technique requires us to convert a ULSS-1 structure into an uncontrolled linear time-varying structure as defined by (1.4.17). Consider the pairs of corresponding system matrices of the LTI subsystems of the ULSS-1 structure, say $\mathbf{A_1}$ and $\mathbf{A_2}$. We wish to use these to form $\mathbf{A}$ as in (1.4.17). We note that in a ULSS-1, an element of some system matrix from the first subsystem can differ from the corresponding element in the corresponding system matrix of the second subsystem. For example, in the structures we consider, such as that in (1.2.11), certain non-zero elements of $\mathbf{A_1}$ correspond to a zero in $\mathbf{A_2}$. In such a case, to form $\mathbf{A}$ as in (1.4.17), we must write a matrix element as a function of the form $\omega(\cdot) = K\left(\mathcal{H}_0(\cdot) - \mathcal{H}_{t_1}(\cdot)\right)$, for K constant, t_1 the switching time, and $\mathcal{H}_0$ and $\mathcal{H}_{t_1}$ particular instances of the Heaviside step function introduced in (1.2.8).

However, the approach of Audoly *et al.* [3] required that expressions for time-varying

[32] The approach employed in Whyte [94] is revised and extended in Section 4.4.

parameters were differentiable with respect to time. As such, it is not applicable to parameters with jumps that result from formulating a ULSS-1 as a linear time-varying system. As Audoly *et al.* [3] is unsuitable for our purposes, we will not consider it further.

The 2014 article, "Identifiability and identification of switched linear biological models" by Guo and Tan [32] considered model structures which were at least similar to those considered in this thesis. The authors considered switched systems for which the system matrix ($\mathbf{A}$) depended on the intensity of some input. In the simplest case, they considered a state-space structure subject to a real, scalar 'pulse excitation' input u, related to state variables $\mathbf{x}$ through

$$\dot{\mathbf{x}} = \mathbf{A}(u)\mathbf{x} + \mathbf{B}u, \quad \mathbf{x} \in \mathbb{R}^n,\ \mathbf{A}(u) \in \mathbb{R}^{n\times n},\ \mathbf{B} \in \mathbb{R}^{n\times 1}, \tag{1.4.18}$$

where

$$u = \begin{cases} u_0, & 0 \leq t \leq t_d, \\ 0, & t > t_d. \end{cases} \tag{1.4.19}$$

The input defined by (1.4.19) is reminiscent of the means of modelling the change of analyte concentration in a structure representing a two-phase biosensor experiment.[33] However, unlike those structures, the type of structure defined by (1.4.18) was controlled. The influence of an input on $\mathbf{A}$ as well as its effect on the state through $\mathbf{B}$ restricted the class of linear switching systems under investigation.

The interest of [32] was in the uniqueness of parameter estimates obtained from data. This made it an inspection of a structure to determine if it was globally *a posteriori* identifiable. The method proposed required numerical data values and was not concerned with whether the property it examined was generic. Hence, there is not an obvious means of adapting it for our goal of testing ULSS-1 structures for global *a priori* identifiability. As such, we obtain no advantage in attempting to reformulate the structures we considered in this thesis to emulate those in Guo and Tan [32].

[33] We will see this in the switching function (3.7.43) used in the description of a ULSS-1 structure given by (3.7.41) in Chapter 3.

Let us recap and relate the literature reviewed above to the motivating application of this thesis: the testing of continuous-time ULSS-1 structures for global *a priori* identifiability where the constituent LSS are composed of compartmental subsystems. Many papers reviewed are not designed for this purpose, for example, as they are intended for structures of discrete-time systems or systems that are not compartmental. Further, these do not actually test a LSS structure for global *a priori* identifiability, or they neglect local *a priori* identifiability, or they make assumptions that we cannot justify for our application.

Aside from these points, most of the methods reviewed are unnecessarily complicated for the ULSS-1 structures we consider as for these it is not necessary to determine the switching time. Some papers intended for time-varying structures are not suited to the analysis of LSS structures. These features suggest that only the methods given in Whyte [90, 91] and [94] — which were originally motivated by our application — are useful for our purposes. We will draw on these papers in developing our approaches to testing a ULSS-1 structure for global *a priori* identifiability in Chapter 4.

Remark 1.3. We have reviewed only those aspects of the literature adjacent to our main problem. However, there are various other threads of research relating to the testing of structures for particular properties. Some of this activity has resulted from the use of large structures (having many parameters and states) to describe complex systems in the field of systems biology. The reader interested in recent developments may wish to consult works such as DiStefano [24] and the references therein.

Another notable review of (and contribution to) the state of the art is given by Villaverde *et al.* [86]. This paper reviewed various software tools for establishing properties of nonlinear structures. It also introduced a new package — STRIKE-GOLDD (STRuctural Identifiability taKen as Extended-Generalized Observability with Lie Derivatives and Decomposition) — for testing structures for the property of local *a priori* identifiability. The software uses a sophisticated iterative scheme that may establish whether individual parameters are locally *a priori* identifiable or *a priori* unidentifiable. The process may ultimately lead to the classification of a structure. Application of the software to test

cases showed it to be both powerful and flexible.

Methods presented in both DiStefano [24] and Villaverde *et al.* [86] were intended for nonlinear structures, but not LSS ones in particular. (For example, Villaverde *et al.* [86] precludes the application of STRIKE-GOLDD to a LSS structure as a result of certain conditions placed on a structure's output function.) Further, it is not immediately apparent how one would modify the methods to make them suited to our context.

Through our focus on testing a LSS structure for global *a priori* identifiability, we have not made a detailed exploration of tests such as those described in Villaverde *et al.* [86]. We may obtain some new insight into our problem by giving greater attention to such works in future studies.

1.5 Prelude to Chapter 2

This research project resulted from the author's time in a laboratory with a Biacore brand flow-cell optical biosensor. These are a popular version of an apparatus that has found widespread use in the monitoring of biomolecular interactions in real time.[34] Experimental studies using Biacore-like biosensors are also well-represented in the literature.[35]

Our primary interest is in the properties of structures employed to model biomolecular interactions studied on such a biosensor. The combination of the type of biomolecular interaction assumed to occur and experimental conditions used dictate the structure employed for this purpose. Typically structures are incompletely specified, which impedes their analysis. As a result, our first concern lies in collecting together information that enables us to produce a completely defined structure for some of the most commonly assumed biomolecular interactions. This assemblage will also inform the process of specifying other

[34] For an overview of various biosensor applications, see, for example, Rich and Myszka [69] and references therein.

[35] This is readily seen from the prominence of Biacore-like optical biosensors in the later annual reviews of the biosensor literature compiled by Rich and Myszka, for example, [68, 69].

structures in future work.

Towards this, Chapter 2 begins with an overview of the experimental apparatus. Interactions occurring in a flow-cell optical biosensor experiment have some similarity to those occurring in a reaction vessel. Following this, we present some preliminary concepts relating to chemical interactions occurring under fixed conditions, and the mathematical modelling of their rates. Through this we produce a sample structure which bears some resemblance to those used to model optical biosensor data. This allows us to show an example of how to test a certain type of structure for global *a priori* identifiability in Chapter 3, following the introduction of some mathematical preliminaries in that chapter.

Some features of interactions occurring on a flow-cell optical biosensor are particular to this context. Appreciating these requires a more detailed discussion of aspects of the operation of the biosensor and experimental conditions. This enables us to model interactions and the resulting data with well-defined ULSS-1 structures. Ultimately these will serve as test cases for our approaches to testing ULSS-1 structures for global *a priori* identifiability.

Chapter 2

An introduction to chemical reactions, flow-cell optical biosensors, and kinetic experiments

2.1 Overview

The experimental system provided by a flow-cell optical biosensor has certain defining features. The system has at least one species immobilised at a sensor chip surface (immobilised ligand) and at least one species in solution (analyte) made to flow over the chip. The biosensor reports a response related to the amount of mass near the chip surface (see, for example, Stenberg *et al.* [75]). Binding of analyte to ligand increases this mass. The sustained increase in response that generally follows[1] indicates the formation of complexes of biomolecular species. This information is obtained without requiring species to have radioactive labels. This, the easy-to-use nature of commercial biosensors, and the high signal-to-noise ratio of data obtained, has led to their use in a range of qualitative and quantitative studies of biomolecular interactions.

[1] A biosensor may only register a change of mass above a certain threshold.

Our interest is in experiments that provide data for the quantification of features of biochemical interactions, such as rate constants. This requires us to represent the experimental system with a model structure that has two key features. The first is a set of expressions for rates of chemical interactions believed to occur. Typically, each of these is a rate of change of some measure of the amount of a chemical species (such as mass or a quantity related to this) that is a function of parameters representing rate constants. The second is a parametric expression relating the quantities modelled by interaction rates to biosensor response.

Some care is needed when modelling an experimental system. Biosensor response is composed of various components, attributable to different sources of mass. Yet only the sum of components related to the forms of analyte-ligand complex indicates the progress of interactions. Attempting to model all components may lead to a structure for which it is not possible to estimate parameters from data. This is an instance of a general problem in which only some function of parameters can be estimated from data, but not the parameters themselves.

Ideally, we would anticipate such an undesirable feature of a structure prior to data collection by testing it for the property of globally *a priori* identifiability. This matter has received little attention in the optical biosensor literature, which tends to focus on the generation and use of biosensor data. Further, structures are commonly presented in an ambiguous manner. That is, the structure may have unstated assumptions, variables that are not completely explained, or it may not specify those components of response that are modelled. Such features can make the relationship of a structure to experimental data unclear. In such a case, scrutiny of structure properties is not necessarily predictive of the value of planned experiments.

This state of affairs encourages us to produce model structures which have an unambiguous meaning. We can achieve this by considering aspects of the experimental system and how these are translated into response. Towards this, we begin by introducing some features of elementary chemical interactions and their mathematical modelling. It

is necessary to adapt aspects of this background to suit the conditions of certain types of flow-cell optical biosensor experiments. Hence, we give a summary of the essential features of these biosensors, as seen in Biacore brand models, and others. We proceed to consider binding experiments, and properties of the reported response. This background provides guidance on how to assemble a well-defined structure representing a given mechanism for biomolecular interactions.

2.2 An introduction to chemical reactions and kinetics

This section gives a brief introduction to chemical reactions and mathematical modelling of reaction rates. The reader requiring further detail may wish to consult a text such as Chang [16].

2.2.1 Irreversible and reversible reactions

A process in which some number of chemical species[2] undergo a chemical change[3] is termed a **chemical reaction**. Examples of chemical changes are the transformation of species into another structural form, or the conversion of some species into others. To give an example of the latter case, consider the burning of molecular hydrogen gas (H_2) in oxygen gas (O_2) to give water (H_2O). Such a reaction is summarised by a **chemical equation**, here

$$2H_2 + O_2 \longrightarrow 2H_2O. \tag{2.2.1}$$

The $\longrightarrow$ shows that (2.2.1) is an **irreversible** reaction, that is, the **reactants** on the left of the arrow are converted into the **products** on the right. Products cannot revert back into reactants under the conditions (such as temperature and pressure) of the reaction. In a chemical reaction matter is not created or destroyed. Hence, the number of atoms of an element present on the left of the arrow must equal the corresponding number on

[2] either elements or compounds

[3] That is, a rearrangement of atoms, rather than a change of physical state, as in when ice melts to become liquid water.

the right to **balance** the equation. This is achieved by choosing an appropriate numerical prefix for each chemical species, as seen in (2.2.1). Typically the smallest possible integer values are used. A balanced equation such as (2.2.1) summarises the **stoichiometry** of the reaction; here, two molecules of diatomic hydrogen gas interact with one molecule of diatomic oxygen gas to produce two molecules of water.

In certain reactions, reactants are not completely converted into products. Consider the interconversion of dinitrogen tetroxide (N_2O_4) and nitrogen dioxide (NO_2):

$$N_2O_4 \rightleftharpoons 2NO_2\,. \tag{2.2.2}$$

The use of $\rightleftharpoons$ in a chemical equation such as (2.2.2) denotes it as a **reversible** reaction. We may think of (2.2.2) as representing two simultaneous reactions whenever both species are present. The **forward reaction**, the conversion of N_2O_4 into NO_2, is seen by reading (2.2.2) from left to right. The **backwards reaction**, the conversion of NO_2 into N_2O_4, is seen by reading (2.2.2) from right to left.

In industrial or biological settings, it is often desirable to understand the progress of a chemical reaction. One way of doing this is to quantify how the amounts of chemical species change over time. However, it is not always possible to measure these amounts as often as required to achieve this. In such a case, we may choose to represent the chemical system with a model structure. The design of such a structure is aided by the introduction of some terminology and definitions. For completeness, these are given in Appendix A. Of these, 'molar concentration' is useful for the description of chemical interactions occurring in a liquid phase. For such interactions, it is common to express the time rate of change of the molar concentration of a species X (say) as a function of the molar concentrations of all species which contribute to the production or consumption of X.

In this thesis we are mostly concerned with reversible interactions. For these, including those studied in certain types of optical biosensor experiments, the long-term behaviour of the interaction is often of particular interest. Associated with this is the degree to which reactants are ultimately converted to products. We will consider the description of this

feature in the next subsection.

2.2.2 Chemical equilibrium

Given a sufficient amount of time, a reversible reaction such as (2.2.2) will reach a state where the amounts of each of the reactants and products do not change. However, the forward and reverse reactions are still occurring. Each species has a rate of consumption that is equal to its rate of production. Such a system is in a state of **dynamic equilibrium**.

Let us consider reactions occurring in solution at a constant temperature as these are appropriate for our application. The molar concentrations of reacting species at equilibrium give a measure of the extent of conversion of reactants into products. To give a simple illustration, consider the balanced reversible reaction for the conversion of A and B to C and D occurring in solution:

$$a\mathrm{A} + b\mathrm{B} \rightleftharpoons c\mathrm{C} + d\mathrm{D}\,. \tag{2.2.3}$$

Let the molar concentrations of A, B, C and D at equilibrium be $[\mathrm{A_e}]$, $[\mathrm{B_e}]$, $[\mathrm{C_e}]$ and $[\mathrm{D_e}]$ respectively. The (always positively-valued) **equilibrium constant** in this case is

$$K \triangleq \frac{[\mathrm{C_e}]^c \cdot [\mathrm{D_e}]^d}{[\mathrm{A_e}]^a \cdot [\mathrm{B_e}]^b}\,, \tag{2.2.4}$$

which has dimensions that depend on the coefficients in (2.2.3).

The meaning of an equilibrium constant is independent of the reaction it applies to. If $K > 1$ ($K < 1$) then at equilibrium the molar concentrations of the products of the forward reaction are greater (less) than those of the reaction's reactants. This disparity grows as K increases (decreases).

2.2.3 Rate of change of molar concentration

Knowledge of a reaction's equilibrium concentrations is insufficient for the estimation of rate constants which characterise the reaction. Such estimation requires both data on the

progress of a chemical reaction over time and a structure to model this. A typical means of achieving the latter is to use a system of ordinary differential equations (ODEs) to model the rates of change of molar concentrations of interacting species.

In order to develop this idea, let c_X denote the molar concentration of species X. The time rate of change of c_X at any instant is the difference between the total rate of processes which increase c_X and the total rate of processes which reduce c_X at that instant.

The rate of increase of c_X is the sum of the rates at which X is formed or arrives in the system, say, $r_{\text{formation}} + r_{\text{arrival}}$. By definition both of these rates are non-negative. The rate of decrease of c_X is the sum of the rates at which X is consumed by a chemical reaction (or decomposes) or is otherwise removed from the system, say, $r_{\text{consumption}} + r_{\text{removal}}$. By definition both of these rates are non-positive. Common units for these rates are moles per decimetre cubed per second (mol dm^{-3} s^{-1}, or equivalently, M s^{-1}). Hence, the overall rate of change of c_X at time t is expressed through a first-order ODE

$$\frac{\mathrm{d}c_X}{\mathrm{d}t}(t) = \underbrace{r_{\text{formation}}(t) + r_{\text{arrival}}(t)}_{\text{total rate of increase of } c_X} + \underbrace{r_{\text{consumption}}(t) + r_{\text{removal}}(t)}_{\text{total rate of decrease of } c_X}, \tag{2.2.5}$$

which has units of mol dm^{-3} s^{-1} as a consequence of its component rates.

Typically, each of the component rates of the right-hand side of (2.2.5) is modelled by a first-order ODE which depends on the molar concentrations of interacting chemical species. The form of an ODE may be suggested by properties of these species or experimental knowledge of the reaction system.

To give a simple example, consider an irreversible reaction occurring under constant experimental conditions (such as temperature and pressure) in solution in a sealed reaction vessel. In such a case we do not need to consider the transport of chemical species into or out of the vessel. For a reaction such as

$$\mathrm{X} \xrightarrow{k} \mathrm{Y}, \tag{2.2.6}$$

k is a fixed positive value termed the **rate constant** of the reaction. A rate constant is a characteristic of a reaction that typically depends on the environmental conditions.

If the rate of consumption of c_X is given by

$$r_{c_X \text{ consumption}}(t) = -k \cdot c_X(t)\,, \tag{2.2.7}$$

then (2.2.6) is a **first-order** reaction. Further, at any point in time the reaction consumes an amount of X and produces an equal quantity of Y, and hence

$$r_{c_Y \text{ production}}(t) = -r_{c_X \text{ consumption}}(t) = k \cdot c_X(t)\,.$$

A form of reaction rate related to (2.2.7) is frequently assumed in the flow-cell optical biosensor literature for certain types of interactions, such as that in which two species bind to form a complex. (For details, see Whyte [97, 99].) These can be combined to give a structure for a system of interactions occurring on a biosensor. We illustrate this with an example of the modelling of multiple interactions in the next subsection. We arrive at a simple example which allows us to demonstrate how to test a model structure for global *a priori* identifiability in Chapter 3.

2.2.4 An example of the modelling of first-order reaction networks

Rates of change of concentrations tend to become more complicated as more chemical species participate in the reaction system. Further, as the number of species increases, the totality of interactions is shown more efficiently as a reaction network rather than by individual reactions. To illustrate this, we draw on Whyte *et al.* [100], an exercise in modelling the conversion of epimers[4] of hopane during pyrolysis[5] of oil-bearing rock. The aim of the study was to determine which of a set alternative structures for the molar concentrations of epimers over time best agreed with experimental data. We proposed one reaction scheme in which reactions converted precursors X and Y into intermediates R and S respectively. We also assumed interconversion between X and Y and between R

[4]Two alternative structural forms of a compound which have the same chemical formula but different structures are termed isomers, of which there are various types. Two epimers are isomers having closely related structures, but the structure of one is not a reflection of the other, as for enantiomers.

[5]heating in the presence of oxygen

and S. Further, we assumed that intermediates R and S decayed to products D_R and D_S respectively.

These assumptions define a reaction network:

$$\begin{array}{ccccc} X & \xrightarrow{k_3} & R & \xrightarrow{k_7} & D_R \\ {\scriptstyle k_2}\uparrow\downarrow{\scriptstyle k_1} & & {\scriptstyle k_6}\uparrow\downarrow{\scriptstyle k_5} & & \\ Y & \xrightarrow[k_4]{} & S & \xrightarrow[k_8]{} & D_S \end{array} . \tag{2.2.8}$$

Each reaction in (2.2.8) shows the conversion of one molecule of some species into one molecule of another.

Testing the structure representing (2.2.8) for global *a priori* identifiability is quite complex, and hence it is not ideal for demonstrating this process. A simplified version — termed Model 0 in Whyte *et al.* [100] — is more suitable. Assuming $k_1 = k_2 = k_5 = k_6 = 0$ in (2.2.8) reduced it to two parallel reactions:

$$\begin{array}{ccccc} X & \xrightarrow{k_3} & R & \xrightarrow{k_7} & D_R \\ Y & \xrightarrow[k_4]{} & S & \xrightarrow[k_8]{} & D_S \end{array} . \tag{2.2.9}$$

We assumed that the rate of decomposition of a species was first-order as in (2.2.7), and that matter could not enter or leave the system.

To build up the ODEs for rates of change of concentrations of species, let us first consider the conversion of X into D_R via R. Let us use notation such as $[X]$ to represent the molar concentration of species X, so that a compound and its concentration are distinct. Then following (2.2.9), the first-order rate of change of $[X]$ is

$$\frac{\mathrm{d}[X]}{\mathrm{d}t} = -k_3[X].$$

Following (2.2.9), the rate of change of $[R]$ is the difference between its rate of increase due to its production from X and its rate of decrease due to conversion into D_R. Under our assumptions and the stoichiometric relationships shown in (2.2.9), this is given by

$$\frac{\mathrm{d}[R]}{\mathrm{d}t} = k_3[X] - k_7[R]. \tag{2.2.10}$$

Finally, the negative of the rate of decomposition of $[R]$ determines the rate of production of $[D_{\mathrm{R}}]$, hence

$$\frac{\mathrm{d}[D_{\mathrm{R}}]}{\mathrm{d}t} = k_7[R]. \tag{2.2.11}$$

The ODEs for molar concentrations of species featuring in the conversion of Y to $\mathrm{D_S}$ in (2.2.9) follow analogously.

Whyte *et al.* [100] assumed that the concentrations of $\mathrm{D_R}$ and $\mathrm{D_S}$ did not influence the other concentrations, and as by-products of the reactions, were not of interest. Let us represent the vector of concentrations of interest by

$$\mathbf{x}(t) \triangleq \begin{bmatrix} [X](t) & [Y](t) & [R](t) & [S](t) \end{bmatrix}^{\top}.$$

Using relationships such as (2.2.10) and (2.2.11), the time rate of change of $\mathbf{x}$ is

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{x}(t) = \begin{bmatrix} -k_3 & 0 & 0 & 0 \\ 0 & -k_4 & 0 & 0 \\ k_3 & 0 & -k_7 & 0 \\ 0 & k_4 & 0 & -k_8 \end{bmatrix} \mathbf{x}(t), \tag{2.2.12}$$

where the unknown initial conditions were modelled in Whyte *et al.* [100] by

$$\mathbf{x}(0) = \begin{bmatrix} X_0 & Y_0 & 0 & 0 \end{bmatrix}^{\top}.$$

The initial concentrations reflect unknown quantities and assumptions made about the physical system. Parameters were used to represent the unknown initial concentrations of X and Y. Initial concentrations of R and S were given as zero as we assumed that X and Y in the rock sample were not converted to other species prior to the application of heat in the experiment.

System (2.2.12) is only part of the representative system of a state-space model structure. Completing this specification requires an expression for the structure's outputs.

In the experiments that obtained data for Whyte *et al.* [100] it was only possible to observe the concentrations of R and S over time. Given this, and defining $\mathbf{y}(t)$ as the vector

of observations associated with (2.2.9) at time $t \geq 0$, $\mathbf{y}$ depends linearly on $\mathbf{x}$ through

$$\mathbf{y}(t) = \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \mathbf{x}(t)\,. \tag{2.2.13}$$

A first-order linear constant coefficient ODE system such as (2.2.12) accompanied by a fixed linear function of its variables as in (2.2.13) is termed a **linear time-invariant state-space system**. A variant of these structures provides an appropriate framework for modelling the dynamics of chemical interactions studied on a flow-cell optical biosensor and the reported response. We will formally present this type of structure in Chapter 3.

The next section introduces important features of optical biosensors in order to inform the modelling of experimental response to follow.

2.3 Features of Biacore flow-cell optical biosensors

We first introduce some terminology to aid the subsequent description of flow-cell biosensor experiments.

Definition 2.1 (Chemical species in an experiment)**. Free immobilised ligand** is not bound to any compound other than the sensor chip surface. **Free analyte** or **free ligand in solution** are not bound to any chemical species. Molecules of any species are **functional** if they are able to bind to an interactant. A **buffer solution** does not contain any interacting species.

The most basic sensor chip consists of a thin gold film on a glass slide. Early sensor chips had a carboxymethyldextran (dextran) matrix bound to this sensor surface. Dextran is described in Myszka [53] (and in similar terms in Karlsson *et al.* [45]) as providing "... a flexible anchor for ligand immobilisation, allowing interactions to occur as in solution" (see Löfas and Johnsson [49] for details). Since this time, a variety of sensor surfaces have been used to facilitate specific applications, see Rich and Myszka [67] for a review.

Interactions between analyte and immobilised ligand occur in the region defined by the matrix bound to the chip [44]. According to Karlsson [41] "The binding events occur in a three-dimensional dextran matrix that extends approximately 100nm out from the sensor surface." We term this region the **reaction volume**.

An integrated fluidics cartridge (IFC) in contact with the sensor chip creates four parallel flow cells numbered from 1 to 4. Pneumatic valves allow the user to direct solutions to a set of consecutively-numbered cells called the **flow path**. Solutions enter the IFC upon being drawn up through a needle that is able to move between multiple solution vessels as required by an experiment. A diagram of the IFC and flow cells is given in Figure 2.3.1.

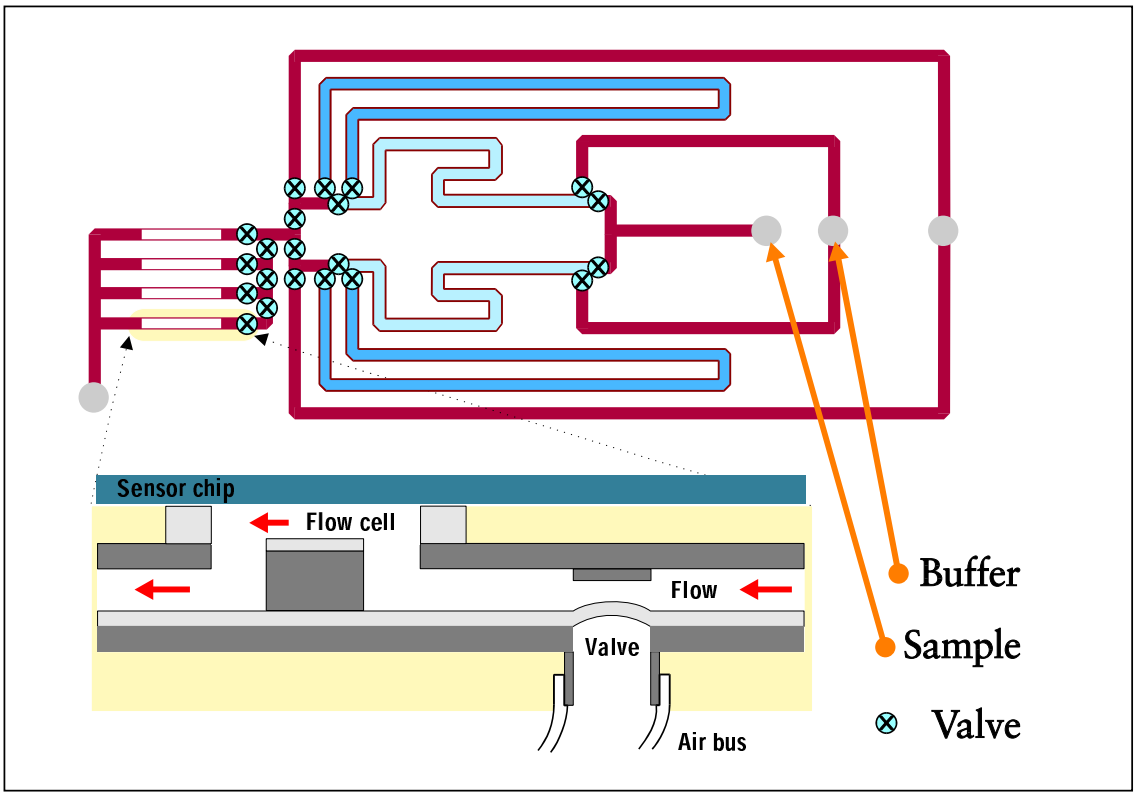


Figure 2.3.1: Biacore flow cells are created by contact between the IFC and sensor chip. (Image from [1]. ©2014 General Electric Company. All Rights Reserved. Reproduced with permission. All GE monogram and GE Healthcare are trademarks of General Electric Company.)

Prior to experiments, at least one cell in the flow path has functional ligand immobilised to its sensor surface via an immobilisation step. This creates a **reaction surface** and the corresponding flow cell is a **reaction cell**. Ligand is immobilised to each sensor surface individually. A **reference cell** either has no ligand or non-functional ligand immobilised to its sensor surface, termed the **reference surface**.

Immobilisation of ligand, and the kinetics of the analyte-ligand interaction in experiments which follow, are quantified as a result of changes in mass in the reaction volume near the sensor surface. A summary of the process for a single flow cell based on [75] follows. For additional information, GE Life Sciences, the parent company of Biacore, have a range of video presentations on the technology, including an introduction [28].

Light from outside of a flow cell passes through a prism and is incident on the glass-gold interface of the sensor chip at a range of angles. This causes an evanescent field to extend from the prism into the gold layer of the sensor chip inside of the flow cell. At a certain angle of incidence, the field couples to an electromagnetic surface wave at the gold-fluid interface. This wave is a 'surface plasmon', and it occurs at the surface plasmon resonance (SPR) angle. The SPR angle is dependent on the mean refractive index of the reaction volume. This is a function of the surface mass concentration. Biacore devices are unable to directly detect an accumulation of mass below approximately 200 Daltons[6] (Myszka [53]).

A detector array measures the intensity of reflected light over an angular range. Light incident at the SPR angle produces a minimum in the reflected light intensity profile. This effect is shown schematically in Figure 2.3.2. The position of this minimum is translated into a signal in response units (RU). Changes in surface mass alter the SPR angle and hence the response value, which is reported against time in a 'sensorgram'.

Prior to an experiment, the sensor chip is exposed to a flow of buffer solution. The RU value averaged over some time window is termed the baseline response of the surface.

[6]One Dalton (equivalently one unified atomic mass unit) is equal to 1/12 of the mass of the carbon-12 atom in its nuclear and electronic ground state.

The surface mass increase resulting from ligand immobilisation increases the response. Subtraction of the baseline from a time average of this response gives a value in RU for the **immobilisation level**.

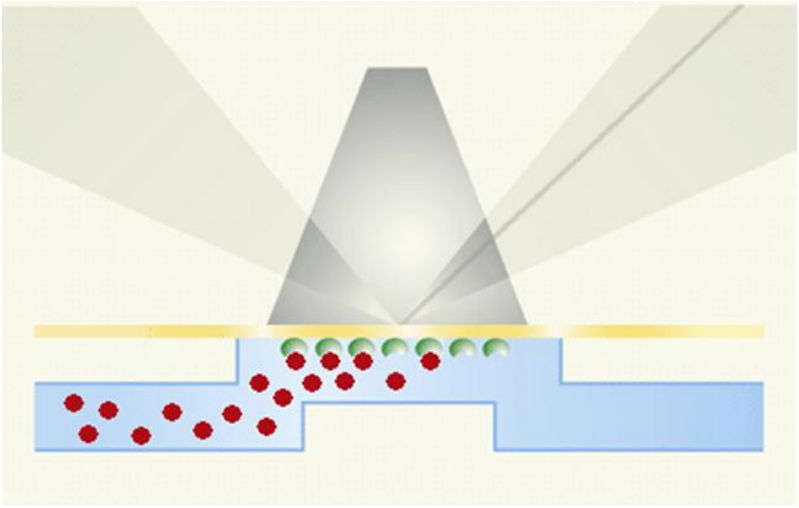


Figure 2.3.2: A schematic of reflected light intensity in a Biacore flow-cell optical biosensor experiment. Light incident on the sensor surface from the left is influenced by mass near the sensor surface. The minimum of reflected light intensity due to SPR is shown as the darker band on the right. Red (green) particles represent analyte (immobilised ligand).

2.4 Biacore experiments

An experiment requires the user to specify a flow path consisting of a reference cell and at least one reaction cell. Beyond this, features of Biacore experiments are dictated by an intended purpose. This section considers experiments where an analyte is known to bind to a particular immobilised ligand.

Our particular interest in this thesis is a particular type of experiment known as a **kinetic experiment**. These aim to obtain response resulting from the interaction of analyte

and immobilised ligand for use in determining the value of rate constants.

2.4.1 Conducting a kinetic experiment

A kinetic experiment consists of a sequence of phases: blank injection, association, dissociation, regeneration, and washing. In **blank injection**, buffer is injected into the flow path. This ensures that the injection needle, IFC and flow path are cleansed of any other solutions.

During the **association phase**, a volume of solution of known analyte concentration is made to flow through the flow path. It is often assumed that analyte concentration is constant in this phase.[7] The net formation of analyte-ligand complex is observed as an increase in sensor response.

The **dissociation phase** begins with a change of the flow solution to buffer. The concentration of analyte in the flow cell decreases to zero over time. Structures representing an experiment performed at a high flow rate commonly assume, often implicitly, that concentration undergoes a step change.[8] Generally there is a net breakdown of complex, leading to freed analyte moving away from the sensor surface and leaving the flow cell. This effect is shown by a decrease in response.

At the conclusion of the dissociation phase, complex may still be present. This complicates the analysis of binding data when a series of consecutive experiments is planned, and is considered undesirable. A **regeneration step** is designed to remedy this. Solutions of a different pH or ionic strength to that of the running buffer are injected into the flow path, promoting complex dissociation. The **washing phase** flushes the solution used in regeneration from the IFC in preparation for the next experimental cycle. A stylized sensorgram showing the effect of the experimental phases on sensor response is given in Figure 2.4.3.

[7] This assumption is known by terms such as the "rapid mixing model", as used in Myszka *et al.* [55].

[8] For example, this condition was assumed in a structure presented in Morton and Myszka [51] and used in the simulations presented in Myszka *et al.* [55].

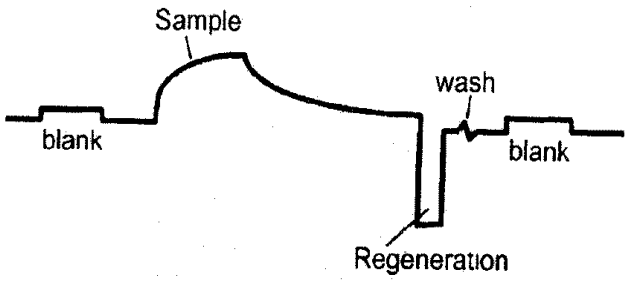


Figure 2.4.3: Typical features of response for phases of a flow-cell optical biosensor kinetic experiment. From Myszka [54], published by John Wiley & Sons Ltd, Baffins Lane Chichester, W Sussex PO19 1UD, England. Reproduced with permission. Copyright ©1999 John Wiley & Sons, Ltd.

Important experimental variables are: immobilisation level, solution flow rate, solution injection volume, analyte concentration, and time allowed for dissociation. The injected analyte volume and flow rate determine the duration of the association phase. The type of experiment dictates an appropriate range of flow rates and immobilisation levels [53]. The response sampling rate can be set to one, five or ten Hz.

For kinetic experiments, Myszka [53] suggested restricting the surface concentration of immobilised ligand to low values and using flow rates at the upper end of the possible range. Under such conditions, response is appropriately modelled as the output of an uncontrolled linear switching system (Whyte [91, 94]).

Remark 2.1. Another common quantitative use of a biosensor is for an **equilibrium experiment**. These share some features with kinetic experiments. However, an equilibrium experiment only has an association phase, which runs until the response reaches a fixed value. This shows that the biomolecular interactions studied have reached equilibrium. The associated response is used for determination of an equilibrium constant (recall Section 2.2.2) rather than rate constants. Modelling the response of such experiments does not require linear switching systems, and hence these are not our main concern in this thesis.

In recent times, some kinetic experiments required the association phase to reach equilibrium before the dissociation phase commenced. We consider this special type of experiment in Chapter 6.

2.4.2 Types of assay

We confine our attention to those types of assay used in kinetic experiments. Karlsson *et al.* [43] defined three such types. The difference between them lies in the nature of the solution injected in the association phase. A **direct binding assay** (DBA) features only one analyte species in the solution (a homogeneous solution). A **surface competition assay** (SCA) has an injected solution containing two analyte species (a heterogeneous solution) that compete for ligand binding sites. An **inhibition in solution assay** (ISA) features a heterogeneous analyte solution in which the two analyte species may form a complex. At least one analyte species is also able to bind ligand when in its free state.

A binding assay typically obtains at least one sensorgram for selected values in a range of analyte concentrations. At least one immobilisation level is used. In SCA and ISA, it is common to use a range of concentrations for one analyte while the other takes a constant concentration throughout an experimental series (see, for example, Karlsson *et al.* [43]). A sensorgram is also collected for a 'blank' (buffer solution without analyte) run. Often the assay is controlled by a computer program into which the user inputs the flow path, experimental variables, and the number of experimental runs (replicates) subject to each set of experimental conditions. The program may randomize the order of injection of the solutions having varying analyte concentrations.[9]

[9]The use of randomization is a tenet of appropriate experimental design. Different types of experiments afford differing means of randomizing experimental conditions. The interested reader may consult a text such as Dean *et al.* [21, Section 1.1.4] for an introduction to the practical and analytical importance of randomization. The first paragraph of Dean *et al.* [21, Section 1.1.4] provides a good summary:

> The purpose of randomization is to prevent systematic and personal biases from being introduced into the experiment by the experimenter. A random assignment of subjects or experimental material to treatments prior to the start of the experimental ensures that the

Figure 2.4.4 presents simulations of response due to complex formation in a SCA series with two species of analyte, A_1 and A_2, of differing molecular mass. The leftmost figure illustrates the decrease in response that results from increased competition for immobilised ligand as the concentration of A_2 increases. The rightmost figure shows some of the variety produced in binding curves by varying analyte concentrations.

Remark 2.2. Experimental conditions in a series of kinetic experiments are chosen to facilitate "accurate determination of rate constants" [8, Page 5-6] from data. This is discussed further in Morton and Myszka [51, Pages 276-277] "When setting up binding experiments, the analyte concentrations should be varied over a wide concentration range. This provides more information about the reaction mechanism." One takes advantage of this information by fitting one model structure to all data, a process termed 'global' fitting or analysis ([51,58]). This achieves "... a better test of the model and improves the statistical behavior of the parameter estimates" ([51]) compared to results obtained by fitting the assumed structure to each curve individually. Software available for global fitting of a structure to data includes CLAMP© [57]. Certain features of this programme were incorporated into an update of the software (starting from BIAevaluation 3.0) accompanying certain Biacore units.

---

observations that are favoured or adversely affected by unknown sources of variation are observations "selected in the luck of the draw" and not systematically selected.

In the context of biosensor experiments, we may introduce systematic error were we to use the same sequence of injected concentrations across the replicates. That is, the order of injected concentrations may unduly influence the time courses of responses we observe. However, (mostly) our mathematical model does not accommodate this feature as it is predicated upon each individual experimental run being independent of any other. Dependencies between runs may impair the ability of our model to approximate data. Randomization of the order of injected concentrations should reduce this systematic error. The literature has commented on this issue. For example, Myszka [54] noted "Randomizing the samples is essential for removing any bias in the data related to when the experiments were carried out." Further, randomisation gives the experimentalist the ability to judge the consistency of biosensor response across replicates of a given injected concentration. Myszka [53] made comment on the usefulness of such a comparison: "Replicated and randomized injections showed the high reproducibility of BIAcore®, even when the binding capacity was very low."

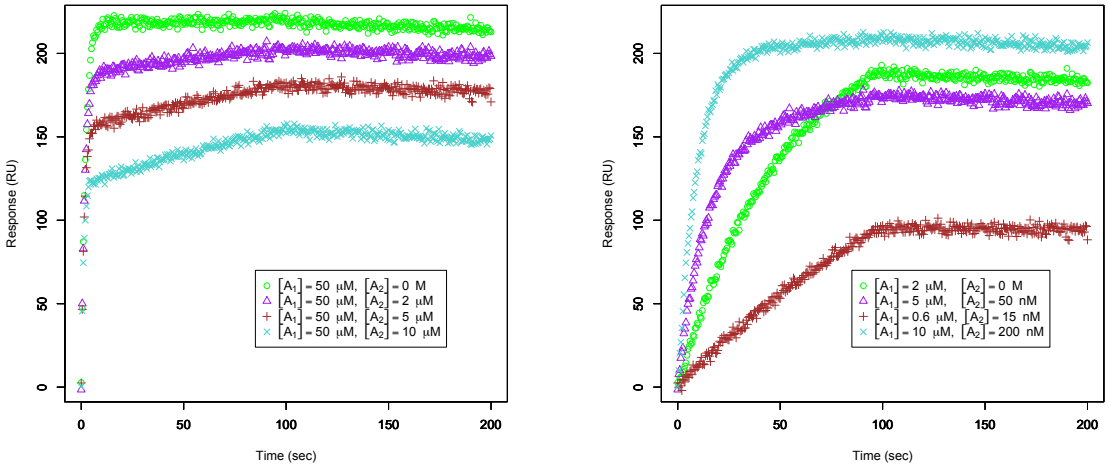


Figure 2.4.4: Simulated response curves for a range of concentrations for the two analyte species A_1 and A_2 in a SCA series where the molecular mass of A_1 is substantially greater than that of A_2. Association and dissociation phases each run for 100 seconds. The assumed interaction mechanism is shown in (6.5.13). Parameter values are as follows: $(k_{a_1}, k_{d_1}, k_{a_2}, k_{d_2}) = (1 \times 10^4, 2 \times 10^{-3}, 4.5 \times 10^4, 8.2 \times 10^{-4})$.

We refer the reader interested in the details of global fitting to the consideration of CLAMP© given in Morton and Myszka [51]. The paper used a diagram to summarise the fitting algorithm. Briefly, CLAMP© uses as inputs the time courses (there may be replicates) of experimental response for each of the employed combinations of experimental conditions. It aims to determine the parameter vector (or vectors) which minimizes a chi-squared (χ^2) value calculated from these inputs and the corresponding model predictions.[10] In order to calculate model predictions, the algorithm first requires the assignment of an initial value to each parameter. Following this we may calculate an initial χ^2 value. The algorithm proceeds by iteratively varying parameter values according to a Levenberg–Marquardt scheme. After each iteration of the algorithm we have a decision point. We

[10]We note that other approaches may use an alternative goodness-of-fit criterion. For example, Joss *et al.* [38] seek to find the parameter vector associated with the best fit of the model to data by minimizing a nonlinear sum of squared errors.

retain any new parameter vector which decreases the χ^2 value relative to its previous value. We use this vector as the initial parameter vector in the next iteration. Conversely, we ignore any new parameter vector which fails to decrease the χ^2 value. Consequently, the current initial vector is reused in the next iteration. The process continues until the reduction in the χ^2 value is smaller than some pre-set threshold. At this point, the last accepted parameter vector is designated as the estimate of the true parameter vector.

In practice, a search algorithm as described may not find a global minimum of χ^2 due to inadequate exploration of the parameter space. For example, the algorithm may become unable to leave a local minimum that is close to the initial parameter vector. For this reason, it is typical to restart the algorithm from a number of randomly generated initial values.

2.5 Remarks on flow-cell biosensor response and interaction models

Typically, a model structure representing an interaction on a flow-cell optical biosensor is not completely specified. In such a case, evaluation of the structure's properties is premature as the results may not have an obvious interpretation. We can overcome this problem by specifying features of the experiment or response to clarify the interpretation of a structure. However, often the literature only suggests to the reader aspects such as the dynamics of the interaction, or which factors contribute to response, or experimental conditions (see, for example, Myszka *et al.* [58], Morton and Myszka [51], and Karlsson and Fält [42]). This is adequate for some situations. For example, often fitting a structure to Biacore data is done by the accompanying BIAevaluation software. In this, the user selects the structure to fit to data by choosing from a menu of interaction models (which includes two interactions considered in Chapter 5). Hence, providing only a standard chemical equation is sufficient to indicate to another user of the software the interaction model used. However, such a minimal amount of information is uninformative when less common structures are used, and is also insufficient for our application.

Specifying the features of an interaction model is challenging due to the variety of alternative formalisms and terminologies seen in the optical biosensor literature. To the best of the author's knowledge, the literature has not attempted to reconcile these. This task was undertaken for the simple bimolecular model in Whyte [99] and for the two-state conformational change model in Whyte [97], and informs the following discussion.

A structure representing an interaction studied by a kinetic experiment on a flow-cell optical biosensor requires certain key features. First is a specification of the experimental conditions. This informs a description of the time behaviour of amounts of chemical species occurring under an assumed interaction. Commonly this is achieved through first-order ODEs for rates of change of variables such as molar concentrations of species.

The second key feature is a relationship for modelling response. There are various sources of mass near the surface in a binding experiment, and each of these contribute to a particular component of the reported response. Only those components due to forms of complex relate to the progress of chemical interactions. However, in certain situations, it may not be possible to process the response to remove extraneous components. When the converse is true, modelling the entire response can lead to a complex model structure having more parameters than are necessary. Recognizing when processing response is possible can lead to a simplified model structure that represents only the informative components. Further, such a simplified structure may be globally *a priori* identifiable when the original structure is not. We will see an example of this in Chapter 5.

Whyte [98] catalogued the components of optical biosensor response. This has aided our interpretation of response representations seen in the literature. Also important for the modelling of response is a relationship between each component and the amount of the species that causes it. O'Shannessy and Winzor [59] asserted such a relationship, which is stated mathematically in the following.

Assumption 2.1 (Response due to a bound chemical species)**.** Suppose X represents either free immobilised ligand or analyte-immobilised ligand complex. Further suppose that the

molecular mass of such a species exceeds the detection limit of the biosensor. In this case, the molar concentration of X, [X] (units M), and the component of response due to X, R_X (in response units, RU) are related by

$$R_X = \rho_X[\mathrm{X}], \tag{2.5.14}$$

where ρ_X has units RU $\cdot$ M^{-1}. The term ρ_X depends on the refractive index increment of X and its molecular mass.

In Whyte [98] the relationship given by (2.5.14) was compared with other response relationships in the literature. It is consistent with Joss *et al.* [38] for the case where response has components due to free immobilised ligand.[11] Equation (2.5.14) also played a useful role in the derivation of response equations seen in the literature from rate equations for interactants (see Whyte [97, 99]).

Rather than recapitulate the synthesis of Whyte [97–99], an assumption and some definitions are reproduced here to aid the interpretation of structures given in Chapter 5. We begin with one useful means of classifying biomolecular interactions occurring on a flow-cell optical biosensor. The classification informs the construction of rate equations for species participating in a biochemical interaction.

Definition 2.2. If the rate of formation of analyte-ligand complex is limited by the rate of diffusion of analyte to the chip surface, it is termed **mass transport limited** (see, for example, Myszka [52]). Otherwise, an interaction is **reaction limited**.

We use the following convention in modelling the basic form of kinetic experiment.

Definition 2.3. A kinetic experiment of two phases consists of an association phase occurring for time $t \in [0, t_1)$ and a dissociation phase[12] occurring for $t \in [t_1, t_f]$, where

[11] By first producing a structure which uses (2.5.14) for response components, we can convert it into a structure for processed response subsequently in some situations.

[12] We defined the association and dissociation phases in Section 2.4.1.

$0 < t_1 < t_f$.

2.6 Prelude to Chapter 3

This chapter has outlined features of biomolecular interactions, flow-cell optical biosensors and certain experiments conducted with them. This will aid the interpretation of model structures representing particular interaction mechanisms that we will see in Chapter 5. Our main interest is in structures that represent an interaction in a kinetic experiment (as outlined in Definition 2.3). These are structures of linear switching systems (LSS). We may think of a LSS as a type of state-space system having piecewise linear time-invariant (LTI) behaviour. We use this feature to inform our approach to testing LSS structures for global *a priori* identifiability, as presented in Chapter 4.

Towards this, Chapter 3 establishes some mathematical preliminaries that are useful in composing our approach. We progress to terminology and definitions applicable to state-space systems. This allows us to define certain structure properties, with particular attention paid to those of LTI state-space structures. A class of these, the compartmental LTI structures, are appropriate for modelling biochemical systems. Particular care is required with their analysis; certain methods suitable for a general LTI structure are unsuitable for the compartmental type. As a result, we collect some properties of compartmental LTI structures and present an established method for testing them for global *a priori* identifiability. We proceed to illustrate the property through an LTI test case.

Chapter 3

Mathematical preliminaries

3.1 Overview

This chapter has two principal aims. The first is to define some types of mathematical systems suitable for modelling the experimental systems introduced in Chapter 2. The second is to present some of their features that will ultimately assist us in defining the property of global *a priori* identifiability for structures of these systems.

Our primary interest is in uncontrolled linear switching systems (ULSSs) as they adequately model the data obtained from a flow-cell biosensor experiment under certain conditions. These systems are piecewise linear time-invariant (LTI). Further, when a structure of ULSSs is an appropriate description of an experimental system, a structure of LTI systems describes the dynamics of any association and dissociation phases. These properties encourage an inspection of LTI systems.

We begin with some preliminaries, such as definitions pertaining to functions. This enables us to define the Laplace transform, an operator particularly well-known for its use in solving linear ODE systems. However, the Laplace transform also gives insight into the parameter information obtainable from the output of the representative system of a LTI structure. This feature makes the Laplace transform a useful tool in the testing of

an LTI structure for global *a priori* identifiability. We will ultimately exploit this in our consideration of ULSS structures.

We proceed to introduce certain classes of system and their properties, and show how a structure may differ from other collections of systems referenced in the literature. We review the key concept of global *a priori* identifiability for a structure of continuous-time systems in generality. Following an introduction to LTI systems, we illustrate the concept of global *a priori* identifiability through an example of testing a specific LTI structure for this property. Finally, we formalise the class of linear switching systems.

We begin our preliminaries by establishing notation.

3.1.1 Notation

If two objects A and B are equivalent, this is expressed by $A \equiv B$.
If A is defined to take the value of B, this is expressed by $A \triangleq B$.

We add some definitions of sets to those employed in Note 1.1. The set of non-negative integers is denoted by $\mathbb{N}_0 \triangleq \mathbb{N} \cup \{0\}$. The subset of $\mathbb{R}$ containing only non-negative values is denoted by $\bar{\mathbb{R}}_+$.

The term $\mathbf{0} \in \mathbb{R}^n$ is the n-tuple consisting of n zero elements. Otherwise, an n-tuple is represented by a bold lower case letter, for example, $\mathbf{x} = (x_1, x_2, \ldots, x_n) \in \mathbb{R}^n$ where $x_j \in \mathbb{R}$ for $j = 1, \ldots, n$. The expression $\mathbf{x} > \mathbf{a}$ where $\mathbf{x}, \mathbf{a} \in \mathbb{R}^n$ is interpreted as $x_j > a_j$, $j = 1, \ldots, n$. The expressions $\mathbf{x} \geq \mathbf{a}$, $\mathbf{x} < \mathbf{a}$ and $\mathbf{x} \leq \mathbf{a}$ are understood in an analogous manner.

Useful subsets of $\mathbb{R}^n$ are $\mathbb{R}^n_+ \triangleq \{\mathbf{x} \in \mathbb{R}^n : \mathbf{x} > \mathbf{0}\}$ and $\bar{\mathbb{R}}^n_+ \triangleq \{\mathbf{x} \in \mathbb{R}^n : \mathbf{x} \geq \mathbf{0}\}$.
Also, for $\mathbf{t} \in \mathbb{R}^n_+$, $\mathbb{R}^n_+(\mathbf{t}) \triangleq \{\mathbf{x} \in \mathbb{R}^n : \mathbf{0} < \mathbf{x} < \mathbf{t}\}$.

For $\mathbf{x}, \mathbf{y} \in \mathbb{R}^n$, the scalar product of $\mathbf{x}$ and $\mathbf{y}$ is $\mathbf{xy} = x_1y_1 + x_2y_2 + \ldots + x_ny_n$. For $\mathbf{x} \in \mathbb{R}^n$, $e^{\mathbf{x}} \triangleq (e^{x_1}, e^{x_2}, \ldots, e^{x_n})$.

At times we need to process a vector (or list of vectors) to produce a vector having

distinct components. This operation is implemented in various programming languages, such as through the "unique" command in R. We can represent this concept mathematically by employing two operations that are informed by our use of Maple to achieve the desired result. For some column vector $\mathbf{a}$, we define the function Υ such that $\Upsilon(\mathbf{a})$ is a set composed of the distinct elements of $\mathbf{a}$. For our purposes, we wish to transform such a set into a vector so that we can refer to elements individually by some index. To achieve this, we define function Ψ such that its action on some set b results in $\Psi(b)$, a column vector with components supplied by b, each component appearing exactly once.

Following the definitions of Υ and Ψ, for some column vectors $\mathbf{a_1}, \ldots, \mathbf{a_k}$ $(k \geq 1)$ we write

$$\langle \mathbf{a_1}, \ldots, \mathbf{a_k} \rangle \triangleq \Psi \left(\bigcup_{i=1}^{k} \Upsilon(\mathbf{a_i}) \right), \tag{3.1.1}$$

which is a column vector of distinct elements drawn from the components of $\mathbf{a_1}, \ldots, \mathbf{a_k}$.

The Kronecker delta function is defined by

$$\delta_{i,j} \triangleq \begin{cases} 1, & i = j, \\ 0, & i \neq j. \end{cases} \tag{3.1.2}$$

Given some scalar valued function $x : t \in T \mapsto x(t) \in X$ (vector valued function $\mathbf{x} : t \in T \mapsto \mathbf{x}(t) \in X^n$, $2 \leq n \in \mathbb{N}$), the first and second derivatives of the function with respect to its argument t are denoted by $\dot{x}$ and $\ddot{x}$ ($\dot{\mathbf{x}}$ and $\ddot{\mathbf{x}}$) respectively. More generally, for $k \in \mathbb{N}_0$, the kth derivative of $\mathbf{x}$ with respect to t is denoted by $x^{(k)}$ ($\mathbf{x}^{(k)}$).

The set of matrices of r rows and c columns ('$r \times c$ matrices', where $r, c \in \mathbb{N}$) having real elements is denoted by $\mathbb{R}^{r \times c}$. A bold upper case letter is used to represent a matrix. For example, consider some matrix $\mathbf{X} \in \mathbb{R}^{r \times c}$ (here $c > r$)

$$\mathbf{X} = \begin{bmatrix} x_{1,1} & x_{1,2} & \cdots & \cdots & x_{1,c} \\ x_{2,1} & x_{2,2} & \cdots & \cdots & x_{2,c} \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ x_{r,1} & x_{r,2} & \cdots & \cdots & x_{r,c} \end{bmatrix}. \tag{3.1.3}$$

One may concisely specify a matrix in terms of elements indexed by row and column labels. For example, $(\mathbf{X})_{i,j} = x_{i,j}$ $(i = 1, \ldots, r,\ j = 1, \ldots, c)$ where $x_{i,j}$ is termed the (i, j)-th element of $\mathbf{X}$.

The transpose of a matrix $\mathbf{X} \in \mathbb{R}^{r\times c}$ is determined by interchanging the rows and columns of $\mathbf{X}$ and is denoted by $\mathbf{X}^\top$. Here, $(\mathbf{X}^\top)_{i,j} = x_{j,i}$ $(i = 1, \ldots, c,\ j = 1, \ldots, r)$ such that $\mathbf{X}^\top \in \mathbb{R}^{c\times r}$.

The square matrices are particularly useful in defining state-space systems. These are a class of matrices in $\mathbb{R}^{r\times c}$ for which $r = c$. For example, consider $\mathbf{A} \in \mathbb{R}^{n\times n}$ $(n \in \mathbb{N})$,

$$\mathbf{A} = \begin{bmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,n} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{n,1} & a_{n,2} & \cdots & a_{n,n} \end{bmatrix}. \tag{3.1.4}$$

The $n \times n$ identity matrix is denoted by $\mathbf{I}_n \in \mathbb{R}^{n\times n}$ where $(\mathbf{I}_n)_{ij} = \delta_{i,j}$ (as in (3.1.2)) for $i, j = 1, \ldots, n$.

A diagonal matrix is specified by the elements on its main diagonal, for example,

$$\operatorname{diag}(\lambda_1, \lambda_2, \lambda_3) \triangleq \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix}.$$

The determinant of an $\mathbb{R}^{n\times n}$ matrix such as $\mathbf{A}$ in (3.1.4) is represented by

$$\det \mathbf{A} \triangleq \begin{vmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,n} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{n,1} & a_{n,2} & \cdots & a_{n,n} \end{vmatrix}.$$

While there is more than one expression for $\det \mathbf{A}$, all are equivalent. For the purpose of illustration, for some $n \geq 2$ and fixed value of $1 \leq i \leq n$

$$\det \mathbf{A} = \sum_{j=1}^{n} a_{i,j} \cdot C_{i,j}\,,$$

where $C_{i,j}$ is the (i,j)-th element of matrix $\mathbf{C}$, the $n \times n$ matrix of cofactors of $\mathbf{A}$.

If it exists, the inverse of some $\mathbf{A} \in \mathbb{R}^{n\times n}$ is denoted by $\mathbf{A}^{-1}$ and it is defined by

$$\mathbf{A}^{-1} = \frac{1}{\det \mathbf{A}} \operatorname{adj} \mathbf{A}\,,$$

where $\operatorname{adj} \mathbf{A} \triangleq \mathbf{C}^\top$ is termed the adjunct of $\mathbf{A}$.

The field of complex numbers is denoted by $\mathbb{C}$. For some $z \in \mathbb{C}$

$$z = x + iy, \quad i^2 = -1,$$

where $\mathrm{Re}(z) = x \in \mathbb{R}$ and $\mathrm{Im}(z) = y \in \mathbb{R}$ denote the real and imaginary part of z respectively.

For $n \in \mathbb{N}$, the set of all n-tuples of complex numbers $\mathbf{z} = (z_1, z_2, \ldots, z_n)$, $z_j \in \mathbb{C}$, $j = 1, \ldots, n$ is denoted by $\mathbb{C}^n$ and termed the n-dimensional unitary space.

We proceed with our presentation of preliminaries by giving definitions relating to functions in the following section.

3.2 Spaces of functions

We begin with an essential definition.

Definition 3.1 (Measurable functions, adapted from [20, Section 2.3])**.** A function f defined on a measurable set Ω in $\mathbb{R}^n$ is **measurable** if the inverse image $f^{-1}(M)$ of any measurable set M in $\mathbb{R}$ is itself measurable. As such, any continuous function is measurable.

Let Ω be an open subset of $\mathbb{R}^n$. The linear space of functions that are continuous on Ω or $\mathbb{R}^n$ are denoted by $C(\Omega)$ or $C(\mathbb{R}^n)$ respectively. The linear space of all measurable functions f (as in Definition 3.1) defined on Ω for which $\int_\Omega |f(\mathbf{x})|^p \mathrm{d}\mathbf{x}$ ($p \in \mathbb{R}$, $p \geq 1$) is finite is denoted by $\mathrm{L}_p(\Omega)$. The space of measurable functions on Ω for which $\operatorname*{ess\,sup}_{\mathbf{x}\in\Omega} |f(\mathbf{x})|$

is finite is denoted by $\mathrm{L}_\infty(\Omega)$. The spaces $\mathrm{L}_p(\Omega)$, $1 \leq p \leq \infty$ equipped with the norms

$$\|f\|_{\mathrm{L}_p(\Omega)} = \begin{cases} \left[\int_\Omega |f(\mathbf{x})|^p \mathrm{d}\mathbf{x}\right]^{1/p}, & 1 \leq p < \infty, \\ \operatorname*{ess\,sup}_{\mathbf{x}\in\Omega} |f(\mathbf{x})|, & p = \infty, \end{cases} \tag{3.2.5}$$

are complete normed spaces, that is, Banach spaces. A shorthand employed by Brychkov *et al.* [12] and duplicated here is $\mathrm{L}_p(\mathbb{R}^n) = \mathrm{L}_p$ and $\|f\|_{\mathrm{L}_p} = \|f\|_p$.

For the discussion of the Laplace transform of a function to follow, it is useful to consider weighted L_p-spaces. For non-negative $\rho \in C(\Omega)$, $\mathrm{L}_p(\Omega;\rho)$ denotes the space of functions defined and measurable on Ω such that $\rho^{1/p} f \in \mathrm{L}_p(\Omega)$. The space $\mathrm{L}_p(\Omega;\rho)$ is a Banach space with the norm

$$\|f\|_{\mathrm{L}_p(\Omega;\rho)} = \|\rho^{1/p} f\|_{\mathrm{L}_p(\Omega)} = \begin{cases} \left[\int_\Omega \rho(\mathbf{x}) |f(\mathbf{x})|^p \mathrm{d}\mathbf{x}\right]^{1/p}, & 1 \leq p < \infty, \\ \operatorname*{ess\,sup}_{\mathbf{x}\in\Omega} \rho(\mathbf{x}) |f(\mathbf{x})|, & p = \infty. \end{cases} \tag{3.2.6}$$

We have now established sufficient fundamental concepts such that we can proceed to define the Laplace transform and some of its key properties.

3.3 Some properties of the Laplace transform

The Laplace transform acts on **original functions**, more succinctly called **originals**. Let us define the properties of the originals of interest to the applications of this thesis. The following is closely based on Brychkov *et al.* [12, Chapter 2].

Definition 3.2. For $\mathbf{a} \in \mathbb{R}^n$, $\mathrm{E}_\mathbf{a}$ is the set of functions from $\mathbb{R}^n$ into $\mathbb{C}$ for which any $f \in \mathrm{E}_\mathbf{a}$ satisfies $f \in \mathrm{L}_1(\mathbb{R}^n_+; e^{-\mathbf{at}})$ (see (3.2.6)), and

$$f(\mathbf{t}) = 0, \quad \mathbf{t} \in \mathbb{R}^n \setminus \bar{\mathbb{R}}^n_+. \tag{3.3.7}$$

That is, $f(\mathbf{t}) = 0$ if at least one component t_j of $\mathbf{t}$ is negative.
Further, $\mathrm{E}_\mathbf{a}$ is equipped with the norm $||f||_{\mathrm{E}_\mathbf{a}} = ||f||_{\mathrm{L}_1(\mathbb{R}^n_+; e^{-\mathbf{at}})}$.

Definition 3.2 assists us in defining the Laplace transform.

Definition 3.3. The (n-dimensional) Laplace transform $\mathcal{L}$ of a function f from $\bar{\mathbb{R}}_+^n$ into $\mathbb{C}$ is defined by

$$F(\mathbf{p}) \triangleq \mathcal{L}\{f\}(\mathbf{p}) \triangleq \int_{\bar{\mathbb{R}}_+^n} e^{-\mathbf{pt}} f(\mathbf{t}) \mathrm{d}\mathbf{t}. \tag{3.3.8}$$

The domain of definition of F is the set of all points $\mathbf{p} \in \mathbb{C}^n$ such that the integral in (3.3.8) is convergent.

Next, we note a consequence of the domain of definition of F given a property of f.

Theorem 3.1. *Define*

$$\mathrm{H}_{\mathbf{a}} = \{\mathbf{p} : \mathbf{p} \in \mathbb{C}^n, \mathrm{Re}(\mathbf{p}) > \mathbf{a}\} \quad \textit{and} \tag{3.3.9}$$

$$\bar{\mathrm{H}}_{\mathbf{a}} = \{\mathbf{p} : \mathbf{p} \in \mathbb{C}^n, \mathrm{Re}(\mathbf{p}) \geq \mathbf{a}\}. \tag{3.3.10}$$

If $f \in \mathrm{E}_{\mathbf{a}}$, then the Laplace integral (3.3.8) *is absolutely and uniformly convergent on $\bar{\mathrm{H}}_{\mathbf{a}}$. Further, F is an analytic function on $\mathrm{H}_{\mathbf{a}}$.*

A consequence of Theorem 3.1 is useful in defining a region on which F is convergent.

Corollary 3.1. *Let $f \in \mathrm{E}_{\mathbf{a}}$ and let the Laplace transform of f denoted by F be convergent at a point $\mathbf{p_0} \in \mathrm{H}_{\mathbf{a}}$. Then it also converges at*

$$\bar{\mathrm{H}}_{\boldsymbol{\sigma}_0} \triangleq \{\mathbf{p} : \mathbf{p} \in \mathbb{C}^n, \mathrm{Re}(\mathbf{p}) \geq \mathrm{Re}(\mathbf{p_0}) = \boldsymbol{\sigma_0}\}.$$

Remark 3.1. For F representing the Laplace transform of original f as defined by (3.3.8), in Brychkov *et al.* [12, Chapter 2] it appears that the phrase "domain of definition of F" used only in Definition 3.3 is equivalent to "the maximal domain $\mathrm{D}(F)$ of convergence of the Laplace integral (2.2) [here this integral is given by (3.3.8)] of f" used subsequently. At other times the term "domain of convergence" is applied to $\mathrm{D}(F)$ or a set such as $\bar{\mathrm{H}}_{\mathbf{a}}$, on which F exists when $f \in \mathrm{E}_{\mathbf{a}}$ (as seen in Theorem 3.1). These two types of domain are not necessarily equivalent.

To avoid possible ambiguity, let us consider 'the domain of convergence of F' to be the unique, maximal domain. Alternatively 'a domain of convergence of F' is not maximal, and further, is not unique. For our purposes in this thesis, a domain of convergence of F as described by Theorem 3.1 is adequate.

The following theorem is useful in determining the Laplace transform of the output functions that arise from the linear switching systems or linear time-invariant systems we employ in this thesis.

Theorem 3.2 (Linearity of the Laplace transform)**.** *Let* $f, g \in \mathrm{E}_{\mathbf{a}}$ *and* $\alpha, \beta \in \mathbb{C}$*. Then* $\alpha f + \beta g \in \mathrm{E}_{\mathbf{a}}$ *and*

$$\mathcal{L}\{\alpha f + \beta g\} = \alpha \mathcal{L}\{f\} + \beta \mathcal{L}\{g\}. \tag{3.3.11}$$

In certain situations we can write (3.3.8) in a form that may simplify its evaluation. If there exists some $\mathbf{p}$ for which (3.3.8) converges and is absolutely convergent then by the Theorem of Fubini,

$$\int_{\bar{\mathbb{R}}_+^n} e^{-\mathbf{pt}} f(\mathbf{t}) \mathrm{d}\mathbf{t} = \int_0^\infty e^{-p_1 t_1} \int_0^\infty e^{-p_2 t_2} \dots \int_0^\infty e^{-p_n t_n} f(\mathbf{t}) \mathrm{d}t_1 \mathrm{d}t_2 \dots \mathrm{d}t_n. \tag{3.3.12}$$

The one-dimensional integrals on the right of (3.3.12) exist, and we may change their order arbitrarily. In this case, we may consider the n-dimensional Laplace transform as a product of one-dimensional Laplace transforms, which are well-tabulated. Brychkov *et al.* [12, Page 85] provides references for the interested reader.

Suppose the n-dimensional Laplace transform (3.3.8) is such that Fubini's theorem is applicable and hence that the factorization (3.3.12) is possible. Brychkov *et al.* [12] draw on the uniqueness theorem for the one-dimensional Laplace transform (see, for example, Doetsch [25]) to give uniqueness theorems for the n-dimensional Laplace transform. One suitable for the purposes of this thesis is reproduced below.

Theorem 3.3 ([12, Theorem 2.6])**.** *If* $\mathcal{L}\{f\} = \mathcal{L}\{g\}$ *on* $\mathrm{H}_{\mathbf{a}}$*, then* $f = g$ *(almost everywhere).*

This introduction to the Laplace transform will prove useful in obtaining features of a LTI structure in Section 3.6.2.4. We will return to this matter after formally defining some classes of system below. These definitions ensure that descriptions of structures we will use later are unambiguous.

3.4 System classes and properties

This section provides definitions of certain classes of systems. The definitions apply to both discrete-time and continuous-time systems, however, our interest is in the latter.

We draw much of the following from Caines [14, Appendix 2], making some minor changes to the text and casting some useful operators in definitions. We let T denote a possibly infinite interval contained in $\mathbb{R}$. Further, let

$$T_+^2 \triangleq \{(t_2, t_1);\ t_2 \geq t_1,\ t_1, t_2 \in T\}. \tag{3.4.13}$$

Other useful notation is u_t to denote the evaluation of a function u at $t \in T$. At times notation such as $[\cdot\,]_t$ is used as an alternative to clarify formulae. We begin by presenting some operators in Definitions 3.4 and 3.5 that are useful subsequently in defining state-space systems, a general class of systems that contains the LSS and LTI systems.

Definition 3.4. Let α be a function mapping T into some set X with an arbitrarily distinguished element x_0. (When X is a linear space we set $x_0 = 0$.) We use P to denote the **(nonanticipative) truncation operator** defined by

$$[P_t\alpha]_w = [P_t\{\alpha_s; s \in T\}]_w = \begin{cases} \alpha_w, & w \leq t;\ w, t \in T, \\ x_0, & w > t;\ w, t \in T. \end{cases}$$

We use Q to denote the **anticipative truncation operator** defined via

$$[Q_t\alpha]_w = [Q_t\{\alpha_s; s \in T\}]_w = \begin{cases} \alpha_w, & w \geq t;\ w, t \in T, \\ x_0, & w < t;\ w, t \in T. \end{cases}$$

Whenever this notation is employed we implicitly assume that the truncated function lies in any given ambient set of functions.

We demonstrate the effects of the non-anticipative and anticipative truncation operators through an example in Figure 3.4.1. This shows that the non-anticipative truncation operator P_t applied to a function only affects the values of the function that occur after time t. Conversely, the anticipative truncation operator Q_t applied to a function only affects the values of the function that occur before time t.

Definition 3.5. For u a function defined on time set T, let S_τ^u denote the **shift operator** where

$$\left[S_\tau^u u\right]_t = u_{t-\tau} \quad \forall t, \tau, t-\tau \in T.$$

We now define some sets and functions which feature in the definitions of various classes of systems. Consider a set of input values U, a set of output values Y and a time set T. Let $\mathcal{U}$ denote a set of input functions such that for $u \in \mathcal{U}, u : T \to U^T : t \mapsto u_t \in U$. That is, $\mathcal{U}$ is a set of input functions taking values in the set U. Similarly, let $\mathcal{Y}$ denote a set of functions such that for $y \in \mathcal{Y}, y : T \to Y^T : t \mapsto y_t \in Y$. That is, $\mathcal{Y}$ is a set of output functions taking values in a set Y. Finally, let ζ denote an **input-output map** from $\mathcal{U}$ to $\mathcal{Y}$. We use these definitions in presenting a general type of system in Definition 3.6. From this definition we may obtain other types of systems by imposing suitable conditions.

Definition 3.6 (Adapted from [14, Definition 1, Appendix 2])**.** An **input-output system** on time set T is a triple $(\mathcal{U}, \mathcal{Y}, \zeta)$.

This system is nonanticipative if, for all $u_1, u_2 \in \mathcal{U}$, and all $t \in T$,

$$P_t u_1 = P_t u_2 \implies P_t y_1 \equiv P_t \zeta(u_1) = P_t \zeta(u_2) \equiv P_t y_2.$$

To explain this further, suppose that T_1 is a subinterval of T for which $\inf T_1 = \inf T$ and $\sup T_1 = t \in T$. Further suppose that inputs u_1 and u_2 are identical on T_1. Then, the

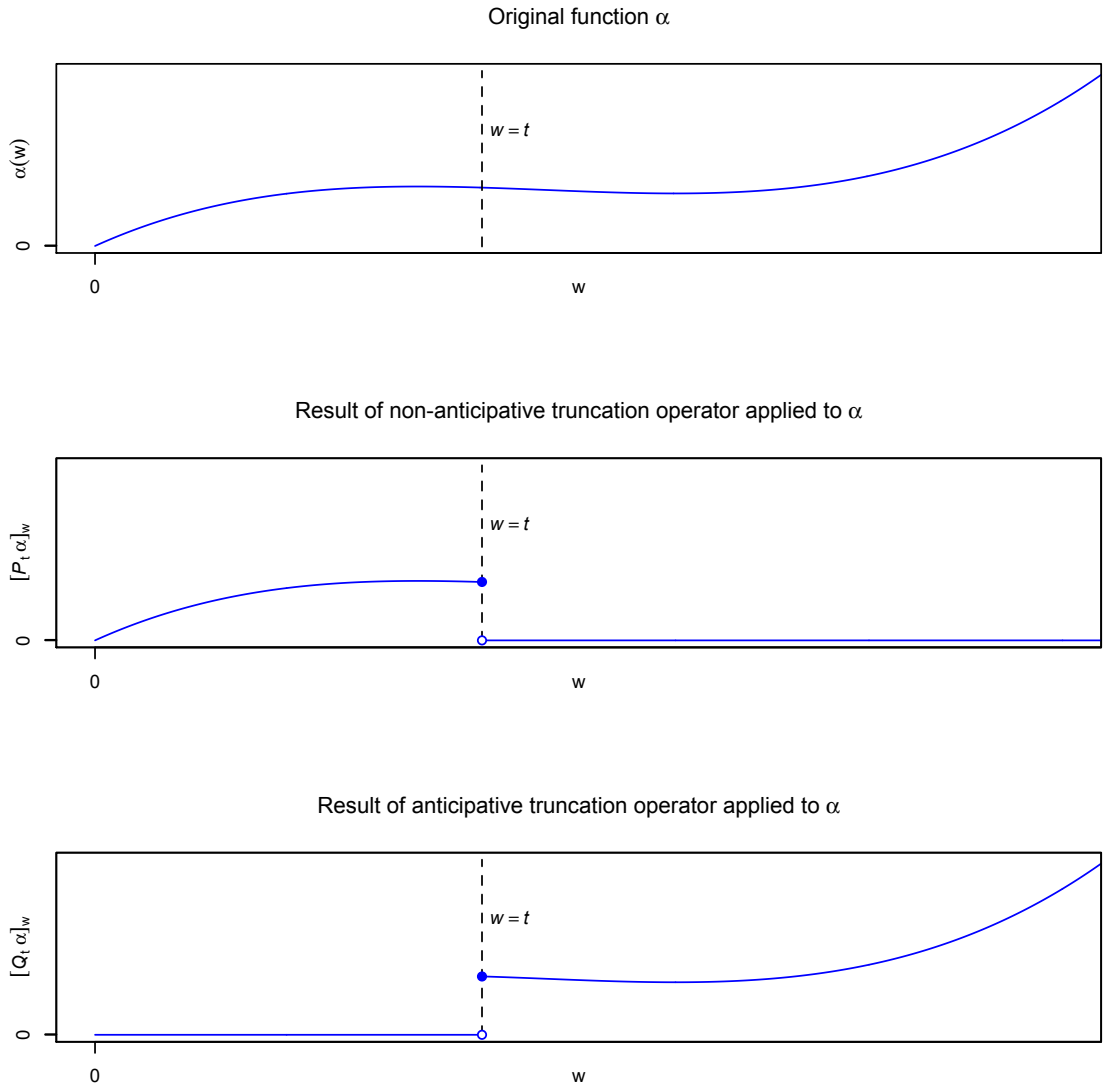


Figure 3.4.1: A sample function and the results of the application of the non-anticipative and anticipative truncation operators (as given in Definition 3.4) to this function.

output functions y_1 and y_2 resulting from the action of the input-output map ζ on u_1 and u_2 respectively are identical on T_1. (Alternatively, when any differences in u_1 and u_2 occur on T only after time t, these do not influence the equivalence of y_1 and y_2 on T_1.) If this property holds for all $t \in T$ then the system is nonanticipative.

Imposing restrictions on U and Y in Definition 3.6 yields a class of system suited to the modelling of many types of physical systems.

Definition 3.7 ([14, Definition 2, Appendix 2])**.** A **(real) linear input-output system** is an input-output system for which the spaces $\mathcal{U}$ and $\mathcal{Y}$ are vector spaces over $\mathbb{R}$, where U and Y are taken to be the real vector spaces $\mathbb{R}^m$ and $\mathbb{R}^k$, respectively, and ζ is a linear map from $\mathcal{U}$ to $\mathcal{Y}$.

The following definition introduces a general class of systems useful for this thesis, making use of T_+^2 as given in (3.4.13). We will obtain systems having particular properties most suited to our application by imposing restrictions on this class subsequently.

Definition 3.8 (Adapted from [14, Definition 4, Appendix 2])**.** A **state-space system** Σ is a quintuple $(\mathcal{U}, X, \mathcal{Y}, \Phi, \eta)$ where

- $\mathcal{U}$ is a set of input functions.
- X is a set, called the state-space of Σ, with elements called states.
- $\mathcal{Y}$ is a set of output functions.
- $\Phi(\cdot\,,\cdot\,,\cdot\,,\cdot\,)$ is the state transition function, which maps $T_+^2 \times X \times \mathcal{U}$ into X.
  To illustrate this, consider time interval $T \subseteq \bar{\mathbb{R}}_+$ with $t_0 \triangleq \inf T$. Suppose Σ is subject to input function $u \in \mathcal{U}$. Further, suppose that at $t = t_0$ we have that $x_0 \in X$ is the initial state of Σ. More generally, for $(t, t_0) \in T_+^2$, $\Phi(t, t_0, x_0, u)$ determines the state of Σ as a consequence of time t, initial state x_0, and input u. Under these conditions, we may concisely refer to $\Phi(t, t_0, x_0, u)$ as the state of Σ at time t.
- $\eta(\cdot\,,\cdot\,,\cdot\,)$ is the output map, which maps $T \times X \times \mathcal{U}$ into Y.
  That is, at some time $t \in T$, η determines the output vector that results from three inputs: t, the state of Σ at that time, and the input u.

Further, the following four properties hold:

SS1: The Identity Property of Φ

$$\Phi(t,t,x,u) = x, \text{ for all } t \in T,\ x \in X \text{ and } u \in \mathcal{U}.$$

That is, suppose the state of Σ at time t is x. Then, if no time has elapsed from t, Φ does not move the state away from x.

SS2: The Nonanticipative Property of Φ

For all $(t_1, t_0) \in T_+^2 \subset \mathbb{R}_+^2$, all $x \in X$, and all $u_1, u_2 \in \mathcal{U}$ we have

$$\Phi(t_1, t_0, x, u_1) = \Phi(t_1, t_0, x, u_2),$$

whenever $P_{t_1} Q_{t_0} u_1 = P_{t_1} Q_{t_0} u_2$.

To explain this, we note that $P_{t_1} Q_{t_0} u_1 = P_{t_1} Q_{t_0} u_2$ requires that the inputs u_1 and u_2 are identical on the time interval $[t_0, t_1]$. Further, suppose the state of Σ at time t_0 is some $x \in X$. Under these two conditions, the Nonanticipative Property of Φ means that Σ reaches the same state at time t_1 for Φ subject to either u_1 or u_2. Equivalently, differences between u_1 and u_2 for any time greater than t_1 do not influence the evolution of the state of Σ on $[t_0, t_1]$ under Φ.

SS3: The Semigroup Property of Φ

For all $(t_1, t_0), (t_2, t_1) \in T_+^2$, $x \in X$, and $u \in \mathcal{U}$,

$$\Phi(t_2, t_0, x, u) = \Phi\big(t_2, t_1, \Phi(t_1, t_0, x, u), u\big).$$

To explain, suppose we have system Σ with initial state x at time t_0 and input u. Suppose Φ acts on time interval $[t_0, t_1]$ resulting in some particular state (say $x_1 \triangleq \Phi(t_1, t_0, x, u)$) at t_1. Suppose then Φ uses x_1 as an initial state for evolving the state of Σ on $[t_1, t_2]$, resulting in a particular state (say $x_2 \triangleq \Phi\big(t_2, t_1, \Phi(t_1, t_0, x, u), u\big)$) at t_2. Due to the Semigroup Property of Φ, system Σ also reaches state x_2 at t_2 if Φ is used to evolve the state on $[t_0, t_2]$.

SS4: The Instantaneous Output Map η

For all $x \in X$, $u \in \mathcal{U}$, $(t, t_0) \in T_+^2$, the function $y : T \to Y$ defined via

$$y(t) = \eta(t, \Phi(t, t_0, x, u), u_t)$$

is a segment of a function in $\mathcal{Y}$.

That is, we can use η to define the instantaneous output of Σ at current time t through t, the state of Σ at time t ($\Phi(t, t_0, x, u)$) and the value of the input at time t (u_t). This property is useful as y provides a simpler means of illustrating the output of Σ than η does when we wish to introduce particular types of system.

We will make extensive use of linear time-invariant state-space systems in modelling individual phases of flow-cell optical biosensor experiments in this thesis. As such, we will proceed to explain the meaning of the descriptors "time-invariant" and "linear" for the context of state-space systems in Definitions 3.9 and 3.10 respectively.

Definition 3.9 ([14, Definition 6, Appendix 2])**.** A state-space system is called **time-invariant** if

1. $\mathcal{U}$ and $\mathcal{Y}$ are closed under translations with respect to the time parameter.
2. $\Phi(t_1+\tau, t_0+\tau, x, S_\tau u) = \Phi(t_1, t_0, x, u)$ for all (t_1, t_0), $(t_1+\tau, t_0+\tau) \in T_+^2$, all $x \in X$ and all $u \in \mathcal{U}$.
3. $\eta(\cdot\,, \cdot\,, \cdot\,) : T \times X \times U \to Y$ is independent of $t \in T$, that is, for all $t+\tau, t \in T$, $x \in X$ and $v \in U$ we have

$$\eta(t+\tau, x, v) = \eta(t, x, v).$$

Definition 3.10 ([14, Definition 7, Appendix 2])**.** A state-space system is a **(real) (finite-dimensional) linear state-space system** if:

1. $\mathcal{U}$ and $\mathcal{Y}$ are vector spaces over $\mathbb{R}$, where U and Y are taken to be the real vector spaces $\mathbb{R}^m$ and $\mathbb{R}^k$ respectively. Further, X is taken to be $\mathbb{R}^n$.
2. For all $(t, t_0) \in T_+^2$, Φ is a linear map of $X \times \mathcal{U}$ into X, that is,

$$\Phi(t, t_0, \alpha x + \beta x', \alpha u + \beta u') = \alpha\Phi(t, t_0, x, u) + \beta\Phi(t, t_0, x', u')$$

for all $\alpha, \beta \in \mathbb{R}$, $x, x' \in X$, $u, u' \in \mathcal{U}$.

3. For all $t \in T$, η is a linear map of $X \times U$ into Y.

In this thesis we are concerned with properties of structures of systems, not merely those of individual systems. We will now draw on the literature in formalising the notion of a structure of systems. This allows us to define a property of a structure useful for a discussion of global *a priori* identifiability. To avoid confusion between our notion of a structure and some competing terminology, we show that they are not equivalent. We then proceed to consider properties of some classes of structure that are useful for the applications we present in this thesis.

3.5 The notions of structure and structured system

3.5.1 Structures revisited

The term 'structure' (sometimes preceded by 'system' or 'model') is commonly used in the field of systems theory to mean a set of parametric systems (or models) of the same type which are related in some specific manner.[1] A structure may not be explicitly defined, in which case it must be inferred from context. Otherwise, the literature defines a structure in different, not necessarily compatible or general ways. In this thesis our interest is in structures of continuous-time state-space systems. We define a general class in the following so that we may later consider structure properties in generality.

Definition 3.11. A **continuous-time state-space system structure** is a collection of continuous-time state-space systems as in Definition 3.8 subject to a particular set of relationships between states, parameters, outputs and (as appropriate) inputs, any constraints on the values of these, and a feasible parameter set.

[1] Recall the introduction to system structure given on Page 2, and the descriptors of types of systems given in Section 3.4.

We have designed Definition 3.11 to avoid the conflation of a structure with its representative system seen at times in the literature. For example, Walter and Pronzato [88, Page 7] discuss structure with reference to a first-order, linear ODE relating output y_m to input u over time by

$$\frac{\mathrm{d}y_m}{\mathrm{d}t} = -p_1 y_m + p_2 u, \quad y_m(0) = 0, \tag{3.5.14}$$

with parameters p_1 and p_2 taken as positive. In describing the structure, the authors state: "One thus defines a class of possible behaviour and a prior feasible set to which the parameter vector $\mathbf{p}$ must belong for the model to be considered acceptable." In this case, we write the parameter vector as $\mathbf{p} \triangleq (p_1, p_2)^\top \in \mathbb{R}^2_+$. We consider an expression such as (3.5.14) to be the representative system of a structure, rather than the structure itself. Further, (3.5.14) is a less general description of a structure compared to that employed in Definition 3.11 as it omits state variables as seen in state-space systems.

Vansteenkiste and Spriet [83] employ a more general description than that used by Walter and Pronzato [88]. The authors considered a general state-space system (which we will interpret as the structure's representative system). The vectors of parameters $\boldsymbol{\theta}$, state variables $\mathbf{x}$, inputs $\mathbf{u}$ and outputs $\mathbf{y}$ are related by

$$\begin{aligned} \dot{\mathbf{x}}(t) = \mathbf{f}(\mathbf{x}, \boldsymbol{\theta}, \mathbf{u}, t), \quad \mathbf{x}(t_0) = \mathbf{x_0}, \\ \mathbf{y} = \mathbf{g}(\mathbf{x}, \boldsymbol{\theta}, \mathbf{u}, t), \\ \mathbf{0} \leq \mathbf{h}(\mathbf{x}, \boldsymbol{\theta}, \mathbf{u}, t), \end{aligned} \tag{3.5.15}$$

where $\mathbf{h}$ represents a vector of constraints on those functions. The authors assert that "Model structure is defined as the functional relationships between the different vectors", here, $\mathbf{x}$, $\boldsymbol{\theta}$, $\mathbf{u}$ and $\mathbf{y}$. They continue with "A model structure is considered here as a class of models", which fails to emphasise that those models are related, as, for example, a collection of LTI systems need not belong to the same structure. However, this usage of "class" could simply expose another instance of a term having a different meaning to different groups. Regardless, the inclusion of states and constraints in Vansteenkiste and Spriet [83] makes their definition more general than others considering input-output systems, and was valuable in informing Definition 3.11.

Other definitions of structure take a different approach to describing features of physical systems. For example, Chestnut [17] considers structure as an aspect of "... the basic information requirements necessary for description of a system". In particular, the structure of a system is defined as "The interrelationships of its parts in one or more respects such as space, time, relative importance, logic or decision-making properties, as influenced by the general characteristics of the whole." Such a definition is much more general than is required for the description of structures of mathematical systems governed by ODEs, for example. However, the philosophy behind Chestnut [17] is more applicable to the process of formulating mathematical models for a physical process.

We will now formalise a feature of the output of structures mentioned in our introduction to global *a priori* identifiability in Section 1.1. We will see later that this feature enables the testing of a structure for certain properties.

3.5.1.1 Observational parameters in structures of systems

Consider a structure M of state-space systems defined for time set T with parameter set Θ as described in Definition 3.8. Consider the structure's representative system $M(\boldsymbol{\theta})$ for some unspecified $\boldsymbol{\theta} \in \Theta$. For various classes of structure[2] we can express the response of $M(\boldsymbol{\theta})$ as

$$\mathbf{y}(t, \boldsymbol{\theta}) = \mathbf{g}(\boldsymbol{\phi}(\boldsymbol{\theta}), t), \quad \forall t \in T. \tag{3.5.16}$$

The $\boldsymbol{\phi}$ in (3.5.16) are termed **observational parameters**, sometimes called **response invariants**, or more succinctly, **invariants**. It may be that different types of invariant vectors can satisfy (3.5.16).[3]

[2] such as LTI state-space structures which we will see in Definition 3.21

[3] We will explain this further in Section 3.6.2.2. The concept is similar to a situation we may observe through representing a suitable function f by more than one type of series expansion. For example, we may take ϕ as defined by the coefficients of monomial terms that constitute a Taylor series representation of f, or alternatively, by the coefficients of sine and cosine functions of a Fourier series representing f.

Regardless of the nature of the invariant vector employed, it must have certain properties. Given an infinite record of error-free data and a particular type of invariant vector, for almost all $\boldsymbol{\theta} \in \Theta$:

- We can exactly determine each element of $\boldsymbol{\phi}(\boldsymbol{\theta})$.
- There is a unique $\boldsymbol{\phi}(\boldsymbol{\theta})$ that satisfies (3.5.16).[4]

We will shortly see in Section 3.5.3 that invariants greatly assist us in testing a structure for global *a priori* identifiability.

The modelling we employ in this thesis has M as some structure of switching systems (as defined shortly in Section 3.7). We cannot write an expression for the response of $M(\boldsymbol{\theta})$ which is as simple as that of (3.5.16) for such a structure. This creates a certain novelty for our application. We shall return to this matter in Chapter 4.

We will now consider an alternative to the term 'structure' that is not suitable for our purposes. However, some of the associated terminology is useful in defining the relationships between systems in a structure.

3.5.2 Structured systems

The term 'structured system' used in the literature actually defines a structure (as defined in this thesis) having a particular property. Thus, a structure is not necessarily a structured system, and so the terms are not equivalent. Terminology to make this distinction clear will be introduced in due course.[5] In order to prevent ambiguity which could occur later, let us first review usages of 'linear structured system'.

[4] This property makes $\boldsymbol{\theta}$ unsuitable as an invariant vector. For $\boldsymbol{\theta}$ to be an invariant vector, it must relate to a structure that is globally *a priori* identifiable, and we do not know this before testing the structure for the property.

[5] That is, in Definition 3.22 following a discussion of linear time-invariant state-space structures.

3.5.2.1 Linear time-invariant structured systems

One opinion on how to define structured systems is given in the following.

Definition 3.12 (Hovelaque *et al.* [35])**.** Linear systems have the form

$$\begin{aligned} \dot{\mathbf{x}}(t) &= \mathbf{A}\mathbf{x}(t) + \mathbf{B}\mathbf{u}(t)\,, \\ \mathbf{y}(t) &= \mathbf{C}\mathbf{x}(t)\,, \end{aligned} \tag{3.5.17}$$

where $\mathbf{x}$(t) is an n-dimensional state vector, $\mathbf{u}(t)$ is an m-dimensional input vector and $\mathbf{y}(t)$ is a p-dimensional output vector. Matrices $\mathbf{A}$, $\mathbf{B}$, and $\mathbf{C}$ are real matrices of appropriate dimensions. These are termed **state-space model matrices**. System (3.5.17) is a **linear structured system** if the entries of the state-space model matrices are either fixed zeros or free parameters.[6]

An alternative description of a linear structured system given in Hovelaque *et al.* [35] is that it is a system as in (3.5.17) "... for which only the existence/absence of a relation between variables is known ...". This definition is not equivalent to Definition 3.12 as it does not require independence of elements of the model matrices.

Poljak [63] considered discrete-time structured systems of the form $\mathbf{x}_{t+1} = \mathbf{A}\mathbf{x}_t + \mathbf{B}\mathbf{u}_t$. Extrapolation of the concepts presented there to continuous-time models follows logically as the definitions relate to the nature of the system parameters, not the manner of time evolution of the state variables. We will provide a useful definition from [63] below, proceeding to give the associated definition of linear structured systems.

Definition 3.13 (Poljak [63])**.** A real matrix $\mathbf{A}$ is said to be **structured** if its nonzero entries are independent variables (or equivalently, algebraically independent reals). This model is suitable in situations where no numerical dependencies among the nonzero entries are expected.

[6] A system described by Definition 3.12 is a particular type of linear state-space system (as in Definition 3.10) that is time-invariant (as in Definition 3.9) system. We will present structures of such systems shortly in Definition 3.21 that we will use subsequently.

Definition 3.14 (Structured time-invariant linear system, Poljak [63])**.** A discrete time-invariant system $\mathbf{x}_{t+1} = \mathbf{A}\mathbf{x}_t + \mathbf{B}\mathbf{u}_t$ is said to be structured if both $\mathbf{A}$ and $\mathbf{B}$ are structured matrices (as in Definition 3.13), and moreover the union of the nonzero entries of $\mathbf{A}$ and $\mathbf{B}$ is algebraically independent as well.

Definition 3.14 is more restrictive than Definition 3.12, and we use the former in subsequent discussion. The matter of whether a structured linear system does or does not have structured matrices is important not merely for classification; it informs how one proceeds to study the properties of the system. For example, in various uses of linear systems to model physical systems, elements of matrices are not independent.[7] Hence, system matrices are not structured matrices as in Definition 3.14. Yamada and Luenberger [101] termed systems with this property 'structured descriptor systems', and recognised that use of the theory of generic controllability[8] intended for structured systems was not appropriate. They continued to propose a method of analysis suitable for the structured descriptor systems.

Remark 3.2. In discussing linear structured systems, Hovelaque *et al.* [35] claim that

> For such systems, one can study generic [or structural] properties, *i.e.* properties which are valid for almost all values of the parameters.

The example given by Yamada and Luenberger [101] shows that one can also study a linear time-invariant structure for which the representative system is not composed of structured matrices for generic properties, albeit in a different manner than one would test a linear structured system.

We continue to consider linear time-varying structured systems in order to demonstrate that the descriptions used are not useful for the linear switching systems of particular interest to us in this thesis.

[7] This is the case for the structures we will study in Chapter 5.

[8] This concept is defined in Section 3.6.3.1.

3.5.2.2 Linear time-varying structured systems

Poljak [63] provided a notion of a structured system for discrete-time time-varying linear systems. The systems have the form $\mathbf{x}_{t+1} = \mathbf{A}_t\mathbf{x}_t + \mathbf{B}_t\mathbf{u}_t$, showing that system matrices may change from one time point to the next. It was assumed that $\mathbf{A}_t \in \mathbb{R}^{n\times n}$ and $\mathbf{B}_t \in \mathbb{R}^{m\times n}$ for $t \in \mathbb{N}_0$. The system matrices have certain properties, which we present below.

Definition 3.15 (Poljak [63])**.** Two matrices $\mathbf{A}$ and $\mathbf{A}'$ are said to be **structurally equivalent** (or to have the same **pattern**), if the nonzero entries of $\mathbf{A}$ and $\mathbf{A}'$ have the same positions (but possibly distinct values).

We draw on Definition 3.15 in defining structured linear time-varying systems below.

Definition 3.16 (Structured linear time-varying systems, Poljak [63])**.** A time-varying system $\mathbf{x}_{t+1} = \mathbf{A}_t\mathbf{x}_t+\mathbf{B}_t\mathbf{u}_t$, is obtained by taking a pair ($\mathbf{A}_t$,$\mathbf{B}_t$) instead of ($\mathbf{A}$,$\mathbf{B}$) [as occur in a time-invariant system] at every step t, where $\mathbf{A}_t$, and $\mathbf{B}_t$, are structurally equivalent to $\mathbf{A}$ and $\mathbf{B}$, respectively, as described in Definition 3.15. We say that a [linear] time-varying system is **structured** if the collection of all nonzero entries of the matrices $\mathbf{A}_0, \ldots, \mathbf{A}_{n-1}$, and $\mathbf{B}_0, \ldots, \mathbf{B}_{n-1}$ is algebraically independent.

Remark 3.3. Definition 3.16 requires a structured system to have a pattern in $\mathbf{A}_t$ and a pattern in $\mathbf{B}_t$ that are each fixed for $t = 0, \ldots, n-1$. This is quite a restrictive condition which is not generally equivalent to that of an uncontrolled LSS structure,[9] and it excludes the LSS structures of interest to us in this thesis. This is as a result of the motivating physical application, in which a kinetic experiment has at least two phases for data collection, say, an association phase and a dissociation phase — recall Section 2.4.1. We will see the incompatibility of structured systems with our structures when we encounter the latter in Chapter 5.

[9] We will meet these in Section 3.7.

We will introduce terminology to clarify the difference between structures and structured systems in Section 3.6.1. This will occur after we have formally defined LTI structures as this will make the distinction more obvious.

The systems theory we have presented in this chapter allows us to progress from the introduction to global *a priori* identifiability of an uncontrolled structure given in Section 1.2 towards a formal definition of the concept.

3.5.3 The generic property of global *a priori* identifiability of an uncontrolled structure

A model structure may have the generic property of global *a priori* identifiability, local *a priori* identifiability, *a priori* unidentifiability, or none of these. Our interest in this thesis is in a test capable of assigning to a structure whichever of these classifications is appropriate. Such a test is known as a test of a model structure for global *a priori* identifiability.[10]

We classify a structure by interpreting the solution set of equations derived from the response of the structure's representative system. In this thesis, the process of classifying an uncontrolled structure draws on definitions of Denis-Vidal and Joly-Blanchard [22], and the modification of Whyte [91] in giving the solution set a name so that we can refer to it subsequently.

Definition 3.17. Consider a structure M of uncontrolled state-space systems with parameter set Θ an open subset of $\mathbb{R}^p$, $p \in \mathbb{N}$, defined for time set $T = [0, t_f) \subseteq \bar{\mathbb{R}}_+$ (as $t_f > 0$) and having state vector $\mathbf{x}(\cdot, \boldsymbol{\theta}) \in \mathbb{R}^n$ and output $\mathbf{y}(\cdot, \boldsymbol{\theta}) \in \mathbb{R}^k$. Its representative system

[10] These tests are distinguished from those tests of a structure for which the best possible classification is as locally *a priori* identifiable.

$M(\boldsymbol{\theta})$, $\boldsymbol{\theta} \in \Theta$, in state-space form is described by relationships of the form

$$\begin{aligned} \dot{\mathbf{x}}(t, \boldsymbol{\theta}) &= \mathbf{f}(\mathbf{x}(t, \boldsymbol{\theta}), \boldsymbol{\theta}), \quad \mathbf{x}(0, \boldsymbol{\theta}) = \mathbf{x}_0(\boldsymbol{\theta}), \\ \mathbf{y}(t, \boldsymbol{\theta}) &= \mathbf{h}(\mathbf{x}(t, \boldsymbol{\theta}), \boldsymbol{\theta}). \end{aligned} \tag{3.5.18}$$

Assume that systems in M satisfy conditions:

1. The functions $\mathbf{f}(\cdot, \boldsymbol{\theta})$ and $\mathbf{h}(\cdot, \boldsymbol{\theta})$ are real and analytic for every $\boldsymbol{\theta} \in \Theta$ on S (a connected open subset of $\mathbb{R}^n$ such that $\mathbf{x}(t, \boldsymbol{\theta}) \in S$ for every $\boldsymbol{\theta} \in \Theta$ and every $t \in [0, \tau]$, $\tau > 0$).

2. $\mathbf{f}\big(\mathbf{x}_0(\boldsymbol{\theta}), \boldsymbol{\theta}\big) \neq \mathbf{0}$ for every $\boldsymbol{\theta} \in \Theta$.

Given $\boldsymbol{\theta} \in \Theta$, finite time $\tau > 0$ and the set

$$\mathcal{I}(M) \triangleq \left\{ \boldsymbol{\theta}' \in \Theta : \mathbf{y}(t, \boldsymbol{\theta}') = \mathbf{y}(t, \boldsymbol{\theta}) \quad \forall t \in [0, \tau] \right\}, \tag{3.5.19}$$

if, for almost all $\boldsymbol{\theta} \in \Theta$,
$\mathcal{I}(M) = \{\boldsymbol{\theta}\}$, M is globally *a priori* identifiable;
the elements of $\mathcal{I}(M)$ are denumerable, M is locally *a priori* identifiable;
the elements of $\mathcal{I}(M)$ are not denumerable, M is *a priori* unidentifiable.

Remark 3.4. Let us consider the implications of Conditions 1 and 2 of Definition 3.17. Part of Condition 1 requires that $\mathbf{f}$ and $\mathbf{h}$ are "analytic". As Condition 1 also requires that these functions are real, let us replace analytic, a term used for complex functions, with differentiable, its equivalent for real-valued functions. This differentiability condition excludes a structure for which any of the state or output functions exhibit jumps.

To appreciate the importance of Condition 2 of Definition 3.17, consider the event where $\dot{\mathbf{x}}(0, \boldsymbol{\theta}) = \mathbf{0}$ for some $\boldsymbol{\theta} \in \Theta$. For an uncontrolled deterministic system, the system state cannot change from the initial state $\mathbf{x_0}(\boldsymbol{\theta})$. (In this case $\mathbf{x_0}$ is an equilibrium state.) As a result, $\mathbf{y}$ — having no explicit dependence on time — is a constant function with $\mathbf{y}(t) = \mathbf{h}(\mathbf{x_0}(\boldsymbol{\theta}), \boldsymbol{\theta})$ $\forall t \in T$. Hence, $M(\boldsymbol{\theta})$ behaves as a degenerate type of system which one would expect provides less parameter information than a typical system where the

output changes with time. Thus, Condition 2 of Definition 3.17 recognizes this problem and excludes such a degenerate system from consideration.

A test of a structure, such as the one we described in Definition 3.17, may also give us information about individual parameters. This may indicate the set of alternative values of a particular parameter that can reproduce the response due to the true parameter vector. The result directs us to assign a description to that parameter that is analogous to the descriptions of a structure we used in Definition 3.17. For example, suppose that, for almost any feasible parameter vector, the only possible value of a parameter is its true value. In such a case, certain authors (such as Walter and Pronzato [88]) judge this parameter to have the property of "parametric identifiability". In order to make such parameter classifications explicit, we present a definition that draws on the formalism we established in Definition 3.17.

Definition 3.18. Suppose in considering structure M we have a particular interest in some particularly important parameter in $\boldsymbol{\theta}$, say θ_i. Further suppose that we require any alternative values of θ_i, say θ_i', that satisfy the equations of the global *a priori* identifiability test. Let us first calculate (3.5.19). If, for almost all $\boldsymbol{\theta} \in \Theta$,
$\mathcal{I}(M)$ shows $\theta_i' = \{\theta_i\}$, then we say θ_i has global *a priori* parametric identifiability;
$\mathcal{I}(M)$ shows that θ_i' has a denumerable set of values, then θ_i has local *a priori* parametric identifiability;
$\mathcal{I}(M)$ shows that θ_i' does not have a denumerable set of values, then θ_i has *a priori* parametric unidentifiability.

Classifying a model structure by application of Definition 3.17 requires solution of the state evolution equation for $\mathbf{x}(\cdot, \boldsymbol{\theta})$ in (3.5.18) to obtain an expression for $\mathbf{y}(\cdot, \boldsymbol{\theta})$, and then finding solutions of functional equations (3.5.19). In some cases it is possible to define global *a priori* identifiability in a different manner that facilitates testing a structure for the property. The representation of a structure's representative system in terms of its

response invariants (Equation (3.5.16)) allows a useful variation on Definition 3.17.

Definition 3.19. Suppose a model structure M with parameter set Θ satisfies the conditions of Definition 3.17. Further suppose that the output of system $M(\boldsymbol{\theta})$ features observational parameters $\boldsymbol{\phi}(\boldsymbol{\theta})$ as described in (3.5.16). For $\boldsymbol{\theta} \in \Theta$, consider the set

$$\mathcal{I}(M, \boldsymbol{\phi}) \triangleq \left\{ \boldsymbol{\theta}' \in \Theta : \boldsymbol{\phi}(\boldsymbol{\theta}') = \boldsymbol{\phi}(\boldsymbol{\theta}) \right\} \equiv \mathcal{I}(M). \tag{3.5.20}$$

It follows that after determining $\mathcal{I}(M, \boldsymbol{\phi})$, M is classified according to Definition 3.17.

Remark 3.5. There are two advantages to using Definition 3.19 rather than Definition 3.17 to test a model structure for global *a priori* identifiability. The first is that the equations in (3.5.19) are replaced by the algebraic equations of (3.5.20), removing the need to solve differential equations. The second is that solving the algebraic equations in (3.5.20) is easier than solving the functional equations in (3.5.19).

We now introduce some other generic properties of a structure that will aid our testing of certain structures for global *a priori* identifiability in Chapter 5.

3.5.4 Other useful generic properties of structures

3.5.4.1 Generic indistinguishability of two structures

Consider structure S_1 (S_2) with parameter set Θ_1 (Θ_2) and output y_1 (y_2). Suppose S_1 and S_2 are each defined for time set $T \subseteq \bar{\mathbb{R}}_+$.

Structure $\boldsymbol{S_1}$ **is generically indistinguishable from** $\boldsymbol{S_2}$ if for almost all $\boldsymbol{\theta}_2 \in \Theta_2$ there exists $\boldsymbol{\theta}_1 \in \Theta_1$ such that $y_1(t, \boldsymbol{\theta}_1) = y_2(t, \boldsymbol{\theta}_2)\ \forall t \in T$.

Structures $\boldsymbol{S_1}$ **and** $\boldsymbol{S_2}$ **are generically indistinguishable** if S_1 is generically indistinguishable from S_2 and S_2 is generically indistinguishable from S_1.

We present an example of two generically indistinguishable structures in Section 3.6.3.4. This will serve to illustrate a useful property of an LTI structure.

3.5.4.2 Generic minimality

When testing a structure for global *a priori* identifiability, the property of generic minimality plays an important — but not necessarily obvious — role. We shall return to this point when considering LTI structures later in this chapter.

Definition 3.20. A structure M composed of systems with state space $X \subseteq \mathbb{R}^n$ and feasible parameter set Θ is **generically minimal** if for almost all $\boldsymbol{\theta} \in \Theta$ (as in Remark 1.2), $M(\boldsymbol{\theta})$ cannot be reduced to a system of $n_1 < n$ states that is indistinguishable (see Section 3.5.4.1) from $M(\boldsymbol{\theta})$.

Recall that Definition 3.11 provided a general notion of system structure. However, showing a system that is representative of its class aids a discussion of the properties of that class. Knowledge of these properties is useful when considering how to test a structure for global *a priori* identifiability. In the following, we will illustrate some types of structures of particular interest to us in this thesis, beginning with some classes of LTI structure.

3.6 Linear time-invariant structures and their properties

3.6.1 Controlled, uncontrolled, and compartmental LTI structures

Definition 3.21 below has its origins in the definition of structured linear time-invariant state-space systems given in van den Hof [81]. The definition given there did not require independence of the nonzero elements of the system matrices. Hence, it is incompatible with the description of a structured linear system given in Definition 3.14. This conflict shows that it is necessary to establish an unambiguous terminology for describing structures. Here we follow the spirit of the definition of van den Hof [81], making some modifications, to define a linear time-invariant structure. We then propose a variant that incorporates the requirements of Definition 3.14 to define a subclass of the linear time-

invariant structures analogous to a linear structured system. The definitions we propose ensure that the meanings of terms used subsequently are clear.

Definition 3.21. A continuous-time linear time-invariant state-space structure (or, more briefly, **an LTI structure**) for given indices n, m and k has a parameter set $\Theta \subset \mathbb{R}^p$ ($p \in \mathbb{N}$) and mappings

$$\mathbf{A} : \Theta \to \mathbb{R}^{n\times n}, \quad \mathbf{B} : \Theta \to \mathbb{R}^{n\times m}, \quad \mathbf{C} : \Theta \to \mathbb{R}^{k\times n},$$
$$\mathbf{D} : \Theta \to \mathbb{R}^{k\times m} \quad \text{and} \quad \mathbf{x_0} : \Theta \to \mathbb{R}^n.$$

These mappings dictate the system matrices in the relationships between state variables $\mathbf{x}$, outputs $\mathbf{y}$, and inputs $\mathbf{u}$ for some unspecified parameter $\boldsymbol{\theta} \in \Theta$ for all times $t \in T$ (where typically time set $T = [0, t_f)$, $t_f \in \mathbb{R}_+$). In particular, the representative system of an LTI structure has the form

$$\begin{aligned} \dot{\mathbf{x}}(t,\boldsymbol{\theta}) &= \mathbf{A}(\boldsymbol{\theta})\mathbf{x}(t,\boldsymbol{\theta}) + \mathbf{B}(\boldsymbol{\theta})\mathbf{u}(t), \quad \mathbf{x}(0,\boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}), \\ \mathbf{y}(t,\boldsymbol{\theta}) &= \mathbf{C}(\boldsymbol{\theta})\mathbf{x}(t,\boldsymbol{\theta}) + \mathbf{D}(\boldsymbol{\theta})\mathbf{u}(t). \end{aligned} \tag{3.6.21}$$

A representative system has a particular pattern of non-zero elements in its matrices, which defines the structure. Systems in the structure have state, input, and output space $X = \mathbb{R}^n$, $U = \mathbb{R}^m$ and $Y = \mathbb{R}^k$ respectively.

There is no requirement for the matrices to be structured in the sense of Definition 3.13.

Defining

$$L\Sigma P(n,m,k) \triangleq \mathbb{R}^{n\times n} \times \mathbb{R}^{n\times m} \times \mathbb{R}^{k\times n} \times \mathbb{R}^{k\times m} \times \mathbb{R}^n,$$

then

$$SL\Sigma P(n,m,k) \triangleq \left\{ \Big(\mathbf{A}(\boldsymbol{\theta}), \mathbf{B}(\boldsymbol{\theta}), \mathbf{C}(\boldsymbol{\theta}), \mathbf{D}(\boldsymbol{\theta}), \mathbf{x_0}(\boldsymbol{\theta})\Big) \in L\Sigma P(n,m,k) \,\Big|\, \boldsymbol{\theta} \in \Theta \right\}$$

describes the set of system matrices of systems belonging to the structure. The matrices of a particular system in this structure are obtained by the parameterisation map $f : \Theta \to$

$SL\Sigma P(n, m, k)$ such that

$$f(\boldsymbol{\theta}) = \Big(\mathbf{A}(\boldsymbol{\theta}), \mathbf{B}(\boldsymbol{\theta}), \mathbf{C}(\boldsymbol{\theta}), \mathbf{D}(\boldsymbol{\theta}), \mathbf{x_0}(\boldsymbol{\theta})\Big).$$

These matrices and vector, and the indices n, m and k, are the **system parameters** of the representative system.

We now define a special class of LTI structures seen in the literature in order to differentiate them from the LTI structures defined previously.

Definition 3.22 (Linear time-invariant I-structure)**.** A LTI state-space structure as exemplified by (3.6.21) is termed a **LTI I-structure** (where I stands for independent matrix elements) if $\mathbf{A}$, $\mathbf{B}$, $\mathbf{C}$, $\mathbf{D}$ and $\mathbf{x_0}$ are structured matrices (as in Definition 3.13), and moreover, the collection of the non-zero entries of these matrices has only algebraically independent elements. A LTI I-structure is equivalent to a linear structured system as in Definition 3.14.

Our primary interest in this thesis is in physical systems that are not subject to inputs. Let us consider a form of LTI structure that is appropriate for modelling such systems.

Definition 3.23. An **uncontrolled linear time-invariant state-space structure** for given indices n and k is effectively a linear time-invariant state-space structure as in Definition 3.21 which does not receive any input. It has a parameter set $\Theta \subset \mathbb{R}^p$ for some $p \in \mathbb{N}$, mappings

$$\mathbf{A} : \Theta \to \mathbb{R}^{n\times n}, \quad \mathbf{C} : \Theta \to \mathbb{R}^{k\times n}, \quad \mathbf{x_0} : \Theta \to \mathbb{R}^n,$$

and representative system $M(\boldsymbol{\theta})$ given by

$$\begin{aligned} \dot{\mathbf{x}}(t, \boldsymbol{\theta}) = \mathbf{A}(\boldsymbol{\theta})\mathbf{x}(t, \boldsymbol{\theta}), \quad \mathbf{x}(0, \boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}), \\ \mathbf{y}(t, \boldsymbol{\theta}) = \mathbf{C}(\boldsymbol{\theta})\mathbf{x}(t, \boldsymbol{\theta}). \end{aligned} \tag{3.6.22}$$

Physical systems – such as those we consider in this thesis – often have inputs, state variables or outputs that are subject to constraints. These may be suitably modelled by variants of LTI structures. Of particular use to us in this thesis are "LTI positive structures" and the "LTI compartmental structures" obtained from these by application of further conditions. We will define these after an overview of their use.

LTI compartmental structures have found widespread use in modelling physiological systems. Some of these systems were studied by introducing a radioactive tracer to an experimental subject and measuring the amounts of tracer in samples drawn from the bloodstream or various tissues (known as compartments) of the subject over time. For these applications, an input was assigned to a particular compartment and an output was the measurement of amount of tracer in an individual compartment. In these and other applications, an uncontrolled LTI structure was appropriate for the first-order chemical reaction schemes considered.[11] System inputs were not required as the system had only impulsive inputs at time zero that were represented as an initial condition.

In defining a compartmental LTI structure, it is appropriate to give a general definition (comparable to that of an LTI structure in Definition 3.21) so that it can accommodate a broader range of applications. The conditions on system matrices in the definition to follow are informed by the definition of compartmental LTI systems given in van den Hof [81].

Definition 3.24. A **positive LTI state-space structure** is an LTI state-space structure having representative system of the form given in (3.6.21) subject to the conditions that the states and outputs of any system in the structure and any inputs to any system are restricted to non-negative values. That is, the structure has $X = \bar{\mathbb{R}}_+^n$, $U = \bar{\mathbb{R}}_+^m$, and $Y = \bar{\mathbb{R}}_+^k$.

A **compartmental linear time-invariant structure** is a positive LTI state-space structure that has system matrices subject to 'conservation of mass' conditions:

- all elements of $\mathbf{B}$, $\mathbf{C}$ and $\mathbf{D}$ are non-negative, and

[11] Recall Section 2.2.3

- for $\mathbf{A} = (a_{i,j})_{i,j=1,\dots,n}$,

$$\begin{aligned} a_{ij} &\geq 0, \qquad && i,j \in \{1,\dots,n\}\,,\ i \neq j, \\ a_{ii} &\leq -\sum_{\substack{j=1 \\ j\neq i}}^{n} a_{ji}, \qquad && i \in \{1,\dots,n\}\,. \end{aligned} \tag{3.6.23}$$

An **uncontrolled compartmental LTI structure** is a compartmental LTI structure which does not feature matrices **B** and **D**.

The conditions of (3.6.23) assign **A** to a particular class of matrices. We will make the properties of such matrices explicit below. These properties determine aspects of the behaviour of LTI compartmental systems and will prove useful shortly.

Definition 3.25 ([73, Section 8.2.1])**.** A square matrix **A** is termed **compartmental** if

1. every off-diagonal element is non-negative,
2. every diagonal element is non-positive, and
3. each column sum is non-positive.

Remark 3.6. Compartmental matrices as in Definition 3.25 are a special case of matrices known by other terms in fields outside of compartmental analysis. Seneta [74] favours the term **ML-matrices** for those matrices which satisfy property 1 of Definition 3.25. Other terms matching this description include **Metzler**, as used in mathematical economics, **essentially non-negative**, and **pseudo-positive**.

The properties required of the set of compartmental matrices shows that they are equivalent to **Minkowski** matrices as presented in Seneta [74], and are a subset of the ML-matrices. Should a compartmental matrix have each column sum equal to zero (a special case of property 3), then it resembles the generator matrix of a Markov chain.

Remark 3.7. Following the notation of Seneta [74], we can relate an ML matrix **A** to a

non-negative matrix $\mathbf{T}$ through

$$\mathbf{T} \triangleq \mu\mathbf{I} + \mathbf{A} \text{ for } \mu \geq 0 \text{ sufficiently large}\,. \tag{3.6.24}$$

When considering Equation (3.6.24), $\mathbf{A}$ is an irreducible matrix (see Seneta [74, Section 1.2]), if $\mathbf{T}$ is irreducible. Such an $\mathbf{A}$ may be termed an **essentially positive** matrix in the numerical analysis literature.

The properties of irreducible ML matrices are considered in Seneta [74, Section 2.3] as extensions of Perron–Frobenius theory of non-negative matrices. Further, ML matrices are considered as a special class of the set of **Perron matrices** Seneta [74, Definition 2.2, Pages 48-49], which makes other results available. To the best of the author's knowledge, results given for compartmental matrices do not explicitly reference this theory.

Remark 3.8. A compartmental LTI structure may not be an LTI I-structure (see Definition 3.22). Specifically, the conditions on $\mathbf{A}$ given by (3.6.23) can lead to a collection of system matrix elements which are not independent. (We will observe this property in the particular compartmental structures we study in Chapter 5.) As a result, certain tools suited to the analysis of I-structures may be inappropriate for compartmental LTI structures; recall the distinction drawn in testing structured descriptor systems for controllability on Page 84.

We note that some popular methods of testing an LTI structure for global *a priori* identifiability depend on first establishing other properties of the structure. Given our remarks above, we may find that such methods are not appropriate for compartmental LTI structures. As a result, we will consider methods for analysing compartmental LTI structures that do not rely on tests we know were intended for I-structures.

3.6.1.0.1 The relation of a compartmental LTI structure to a chemical system We will consider structures representing biochemical reactions (as introduced in Chapter 2) in Chapter 5. The following remarks aim to illustrate features of a compartmental structure or compartmental system used to describe a chemical system. Remarks on compartmental

systems apply whether they belong to an LTI structure or a linear time-varying structure. We consider this latter case in more detail in Section 3.7.

This discussion will embellish upon the introduction to compartmental systems given in Section 1.1. Each compartment of a compartmental system represents a chemical species in a particular location. For example, in considering an experimental subject, glucose in the bloodstream and glucose in muscle may be treated as two distinct compartments. The state variable associated with a compartment gives a measure of the amount of the species in the particular compartment, commonly, some form of concentration (see Appendix A). Exchange of material between compartments is caused by two types of processes. The first are chemical reactions which convert one species (or one conformation of a species) into another. The second type of process is transport, where a species moves from one region to another distinct region, such as from muscle to the bloodstream in the example given above. Material may also be lost from the system by a flow from a compartment to a region outside of the collection of compartments. This is termed an excretion to the environment.

When choosing to model a physical system with a linear compartmental structure, we incorporate *a priori* information or beliefs about the system into the structure as conditions on the elements of system matrices which define the structure. In modelling a chemical system, let us consider the matrices in the most general type of representative system of an LTI structure shown in (3.6.21). For $\mathbf{A}$, $a_{i,j} \neq 0$ for $i \neq j$ allows a flow of matter from compartment j to compartment i. For $\mathbf{B}$, $b_{i,j} \neq 0$ shows that input j is received by compartment i. For $\mathbf{C}$, $c_{i,j} \neq 0$ shows compartment j contributes to output i. For $\mathbf{D}$, $d_{i,j} \neq 0$ shows that input j contributes directly to output i.

The nature of flow-cell optical biosensor experiments directs us to a particular interest in uncontrolled compartmental LTI structures. Let us consider attributes of representative systems from such structures. This will aid our analysis of structures in Chapter 5.

3.6.2 Properties of the state vector and response of uncontrolled LTI systems

3.6.2.1 The solutions for state and response of an uncontrolled LTI system

A system such as (3.6.22) has a form of solution which will prove useful later. The solution for the state vector of (3.6.22) is

$$\mathbf{x}(t,\boldsymbol{\theta}) = e^{\mathbf{A}(\boldsymbol{\theta})t}\mathbf{x_0}(\boldsymbol{\theta}), \tag{3.6.25}$$

from which it follows that the solution for the output vector is

$$\mathbf{y}(t,\boldsymbol{\theta}) = \mathbf{C}(\boldsymbol{\theta})e^{\mathbf{A}(\boldsymbol{\theta})t}\mathbf{x_0}(\boldsymbol{\theta}). \tag{3.6.26}$$

Writing (3.6.25) in another form makes certain features of the time course of $\mathbf{x}$ and $\mathbf{y}$ more apparent. This is achieved by employing the spectral decomposition of $\mathbf{A}(\boldsymbol{\theta})$, (see, for example, [73]), which exists when $\mathbf{A}(\boldsymbol{\theta})$ has n linearly independent right eigenvectors.[12] In this case, the spectral decomposition of $\mathbf{A}(\boldsymbol{\theta})$ gives

$$\mathbf{A}(\boldsymbol{\theta}) = \mathbf{S}(\boldsymbol{\theta})\boldsymbol{\Lambda}(\boldsymbol{\theta})\mathbf{S}^{-1}(\boldsymbol{\theta}), \tag{3.6.27}$$

where $\boldsymbol{\Lambda}(\boldsymbol{\theta})$ is a diagonal matrix of the eigenvalues $\lambda_1(\boldsymbol{\theta}), \ldots, \lambda_n(\boldsymbol{\theta})$ (not necessarily distinct) of $\mathbf{A}(\boldsymbol{\theta})$, $\mathbf{S}(\boldsymbol{\theta})$ is the matrix of right eigenvectors of $\mathbf{A}(\boldsymbol{\theta})$, where the i-th column $(i = 1, \ldots, n)$ of $\mathbf{S}(\boldsymbol{\theta})$ is the eigenvector $\mathbf{s}_i(\boldsymbol{\theta})$ which is associated with $\lambda_i(\boldsymbol{\theta})$. The matrix $\mathbf{S}^{-1}(\boldsymbol{\theta})$ is the inverse of $\mathbf{S}(\boldsymbol{\theta})$ and its rows are the left eigenvectors of $\mathbf{A}(\boldsymbol{\theta})$. We define $\mathbf{s}^{(i)'}(\boldsymbol{\theta})$ as row i of $\mathbf{S}^{-1}(\boldsymbol{\theta})$.

Using (3.6.27), $\mathbf{A}^m(\boldsymbol{\theta}) = \mathbf{S}\boldsymbol{\Lambda}^m(\boldsymbol{\theta})\mathbf{S}^{-1}(\boldsymbol{\theta})$ for $m \in \mathbb{N}$ and hence

$$e^{\mathbf{A}(\boldsymbol{\theta})t} = \mathbf{S}(\boldsymbol{\theta})e^{\boldsymbol{\Lambda}(\boldsymbol{\theta})t}\mathbf{S}^{-1}(\boldsymbol{\theta}), \quad \text{where } e^{\boldsymbol{\Lambda}t} = \operatorname{diag}(e^{\lambda_1(\boldsymbol{\theta})t}, \ldots, e^{\lambda_n(\boldsymbol{\theta})t}).$$

[12] A more generally applicable matrix decomposition is the Jordan decomposition as this applies to any square matrix with complex entries. This decomposition applied to matrix $\mathbf{A}(\boldsymbol{\theta})$ utilises an expression such as $\mathbf{A}(\boldsymbol{\theta}) = \mathbf{P}^{-1}(\boldsymbol{\theta})\mathbf{J}(\boldsymbol{\theta})\mathbf{P}(\boldsymbol{\theta})$ for $\mathbf{J}(\boldsymbol{\theta})$ a matrix in Jordan canonical form and $\mathbf{P}(\boldsymbol{\theta})$ a matrix having linearly independent columns that are elements of a Jordan basis for $\mathbf{A}(\boldsymbol{\theta})$. Using the Jordan decomposition to make an argument similar to the following requires more notational complexity than the use of the spectral decomposition, and this latter decomposition is adequate for our purposes in this thesis.

For simplicity, let us consider the case where $\mathbf{A}(\boldsymbol{\theta}) \in \mathbb{R}^{n\times n}$ has n distinct eigenvalues. In this case we have an alternative expression for (3.6.25):

$$\mathbf{x}(t,\boldsymbol{\theta}) = e^{\mathbf{A}(\boldsymbol{\theta})t}\mathbf{x_0}(\boldsymbol{\theta}) = \sum_{i=1}^{n} \mathbf{v}_i(\boldsymbol{\theta}) e^{\lambda_i(\boldsymbol{\theta})t}, \quad \text{where} \quad \mathbf{v}_i(\boldsymbol{\theta}) \triangleq \left(\mathbf{s}^{(i)\prime}(\boldsymbol{\theta})\mathbf{x_0}(\boldsymbol{\theta})\right)\mathbf{s}_i(\boldsymbol{\theta})\,. \tag{3.6.28}$$

One may inspect the properties of the output of an uncontrolled LTI structure's representative system by considering the Laplace transform of that output. The following remark introduces a topic which we shall return to later in this chapter.

Remark 3.9 (Domain of convergence for $\mathcal{L}\{y(\cdot,\boldsymbol{\theta})\}(s)$ in an uncontrolled LTI structure)**.** The largest eigenvalue of $\mathbf{A}$, say λ_1, effectively determines the domain of convergence of $\mathcal{L}\{y\}$. Suppose λ_1 has multiplicity $\mu \geq 1$. The time-varying term corresponding to λ_1 in y is $t^{\mu}e^{\lambda_1 t}$. For $\mathbf{A}$ a $n \times n$ matrix of finite elements where n is finite, the eigenvalues of $\mathbf{A}$ are finite. Hence, there exists some constants K and λ such that $|y(t,\boldsymbol{\theta})| \leq Ke^{\lambda t}$ for all $t \in \bar{\mathbb{R}}_+$. As a result, $\mathcal{L}\{y(\cdot,\boldsymbol{\theta})\}(s)$ exists for all $s \in \mathrm{H}_\lambda$ (recall (3.3.9)).

3.6.2.2 Observational parameters in LTI structures

Recall the notion of observational parameters of the representative system of a structure as described by Equation (3.5.16). Consider a time-invariant state-space system as described in Definitions 3.8 and 3.9, defined for time set T. For such a system, we can define the $\boldsymbol{\phi}$ in (3.5.16) in different ways. The formulation below has found use in testing uncontrolled LTI state-space structures for global *a priori* identifiability, and controlled LTI state-space structures for system identifiability, as described on Page 25.[13]

[13]These two cases are similar as in each type of test one is able to access the invariants which characterise the response of the structure's representative system.

3.6.2.3 Markov and initial parameters of controlled LTI structures

Consider LTI structure M of n states, k outputs, m inputs, having parameter set Θ and representative system $M(\boldsymbol{\theta})$ as in (3.6.21) of Definition 3.21.

The **Markov parameters** of $M(\boldsymbol{\theta})$ are denoted by

$$M(j, \boldsymbol{\theta}) \triangleq \mathbf{C}(\boldsymbol{\theta})\mathbf{A}(\boldsymbol{\theta})^j\mathbf{B}(\boldsymbol{\theta}) \in \mathbb{R}^{k\times m}, \quad j \in \mathbb{N}_0. \tag{3.6.29}$$

The **initial parameters** of $M(\boldsymbol{\theta})$ are

$$\begin{aligned} N(0, \boldsymbol{\theta}) &\triangleq \mathbf{0} \in \mathbb{R}^k, \\ N(j, \boldsymbol{\theta}) &\triangleq \mathbf{C}(\boldsymbol{\theta})\mathbf{A}(\boldsymbol{\theta})^{j-1}\mathbf{x_0}(\boldsymbol{\theta}) \in \mathbb{R}^k, \quad j \in \mathbb{N}. \end{aligned} \tag{3.6.30}$$

The initial parameters are all zero vectors in the case where $\mathbf{x_0}(\boldsymbol{\theta}) = \mathbf{0}$.

As a consequence of the Cayley–Hamilton Theorem, an LTI system of n state variables has at most $2n - 1$ independent Markov parameters ([79]). This constraint also applies to the system's initial parameters.

Hence, the output of $M(\boldsymbol{\theta})$ is summarised by the vector of observational parameters

$$\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq \left(M(1, \boldsymbol{\theta})^\top, \ldots, M(2n-1, \boldsymbol{\theta})^\top, N(1, \boldsymbol{\theta})^\top, \ldots, N(2n-1, \boldsymbol{\theta})^\top\right)^\top.$$

We will now consider the notion of observational parameters for uncontrolled LTI systems.

3.6.2.4 Observational parameters in uncontrolled LTI systems

3.6.2.4.1 Laplace transform coefficients Consider an uncontrolled compartmental LTI structure as in Definition 3.24 defined for time set $T = \bar{\mathbb{R}}_+$ with $X = \bar{\mathbb{R}}_+^n$ and $Y = \bar{\mathbb{R}}_+$. The Laplace transform of the scalar response y,

$$\mathcal{L}\{y(\cdot, \boldsymbol{\theta})\}(s) = \mathbf{C}(\boldsymbol{\theta})(s\mathbf{I}_n - \mathbf{A}(\boldsymbol{\theta}))^{-1}\mathbf{x}(0, \boldsymbol{\theta}), \quad s \in \mathbb{C}_0, \tag{3.6.31}$$

is a rational function in s, and $\mathbb{C}_0 \subseteq \mathbb{C}$ is termed the domain of convergence of $\mathcal{L}\{y(\cdot, \boldsymbol{\theta})\}(s)$. An expression such as (3.6.31) is henceforth known as the **unprocessed Laplace transform of response.** By Remark 3.9, it is certain that $\mathcal{L}\{y(\cdot, \boldsymbol{\theta})\}(s)$ exists on some domain.

Definition 3.26 (The canonical form of $\mathcal{L}\{y\}$ obtained from an uncontrolled LTI structure)**.** Consider an uncontrolled LTI structure with $X = \bar{\mathbb{R}}_+^n$ and $Y = \bar{\mathbb{R}}_+$, having a representative system that is a modified form of that shown in Definition 3.24 as it omits terms relating to an input $\mathbf{u}$. The unprocessed $\mathcal{L}\{y(\cdot, \boldsymbol{\theta})\}(s)$ for this representative system has the form given in (3.6.31). It is written in a **canonical form** by

- cancelling any common factors between the numerator and denominator, and
- ensuring that the coefficient of the highest power of s in the denominator is 1.

For a suitable domain of convergence $\mathbb{C}_0$, this gives an expression of the form

$$\begin{aligned} \mathcal{L}\{y(\cdot, \boldsymbol{\theta})\}(s) = \frac{\phi_{r+p}(\boldsymbol{\theta})s^p + \cdots + \phi_r(\boldsymbol{\theta})}{s^r + \phi_{r-1}(\boldsymbol{\theta})s^{r-1} + \cdots + \phi_0(\boldsymbol{\theta})}, \quad \forall s \in \mathbb{C}_0, \\ r \in \{1, \ldots, n\}, \quad p \in \{0, \ldots, r-1\}. \end{aligned} \tag{3.6.32}$$

Given the Laplace transform of the output of an uncontrolled LTI system in canonical form as in Equation (3.6.32), the $\phi_i(\boldsymbol{\theta}) \not\equiv 0$, $i = 0, \ldots, r+p$ provide a set of response invariants. These will play a vital role in our formulation of methods for testing an LSS structure for global *a priori* identifiability in Chapter 4.

Remark 3.10. Comparison of the factored form of each of the numerator and denominator of $\mathcal{L}\{y(\cdot, \boldsymbol{\theta})\}(s)$ can show if there are common factors which require cancellation in order to obtain the canonical form. Following any necessary cancellation, the numerator and denominator of $\mathcal{L}\{y\}(s)$ are relatively prime for almost all $\boldsymbol{\theta} \in \Theta$.

3.6.2.4.2 Initial parameters Recall the initial and Markov parameters of a controlled LTI system $M(\boldsymbol{\theta})$ given in Section 3.5.1.1. The Markov parameters as shown in (3.6.29) are all null for an uncontrolled LTI system. The initial parameters of $M(\boldsymbol{\theta})$ are as shown in

(3.6.30). Hence, the output of uncontrolled LTI system $M(\boldsymbol{\theta})$ may be expressed a function of the observational parameters

$$\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq \left(N(1,\boldsymbol{\theta})^\top, \ldots, N(2n-1,\boldsymbol{\theta})^\top\right)^\top.$$

3.6.2.5 Properties of eigenvalues of compartmental LTI systems

Matrix $\mathbf{A}$ as shown in (3.6.21) contributes to defining an LTI structure, and eigenvalues of $\mathbf{A}$ influence the behaviour of systems in that structure. When $\mathbf{A}$ is a compartmental matrix, its eigenvalues have certain properties.

Remark 3.11 (Seber and Wild [73, Section 8.3.3])**.** For $\mathbf{A}$ a compartmental matrix as described in Definition 3.25, $\mathbf{A}$ has no purely imaginary eigenvalues, and the real part of any eigenvalue is non-positive.

Remark 3.12. Given an irreducible ML matrix $\mathbf{B}$, Theorem 2.6 of Seneta [74] guarantees the existence of an eigenvalue τ that is real and is greater than the real part of any other eigenvalue of $\mathbf{B}$. Other conditions are provided such that one can infer either non-negativity or negativity of τ.

Alternatively, Theorem 2.8 of Seneta [74] indicates that for a Perron matrix (recall Remark 3.7) $\mathbf{A}$ there exists some eigenvalue τ that is real. Corollary 1 that follows gives an upper and lower bound on τ through the maximum and minimum column sums of $\mathbf{A}$. If $\mathbf{A}$ is also a compartmental matrix for which all column sums are zero, this shows that $\tau = 0$.

Seber and Wild's result as presented in Remark 3.11 gives us information about τ from a compartmental matrix even if the matrix is reducible. We saw one such matrix in our illustration of the modelling of a chemical reaction system in Equation (2.2.9).[14] This shows at least one situation in which Seber and Wild's results are particularly suited

[14] We can show that the matrix is reducible as reordering the state variables by simultaneous permutations of the rows and columns yields a block upper triangular matrix.

to the analysis of compartmental models. However, we also note that for an irreducible compartmental matrix $\mathbf{A}$ with all column sums equal to zero, Seneta's results shown above combine to give $\tau = 0$. That is, Seneta's results imply the result of Seber and Wild on the non-positivity of the real part of all other eigenvalues of $\mathbf{A}$.

A further exploration of Perron–Frobenius theory may illustrate other properties of compartmental matrices that are useful for analysis of the type we undertake in this thesis. We will consider this in further studies.

We now continue to consider certain properties of LTI structures that inform the test of such a structure for global *a priori* identifiability.

3.6.3 Some generic properties of LTI structures

Recall the statement of a generic property of a structure given in Definition 1.2. In this section we present some generic properties of LTI structures or LTI I-structures. These properties may occur in association with a discussion of global *a priori* identifiability of such a structure. We consider properties of I-structures including controllability (Section 3.6.3.1); its generalisation, weak reachability (Section 3.6.3.2); and observability (Section 3.6.3.3). Given Remark 3.8, we require a method able to test an uncontrolled compartmental LTI structure for global *a priori* identifiability that does not depend on those properties. The discussion to follow ultimately informs our analysis of the structures of interest to our application; those composed of uncontrolled linear switching systems which have piecewise compartmental LTI behaviour.

3.6.3.1 Generic controllability

Lin [47] is often cited for the contribution of generic (structural) controllability to systems theory. From the assumption of independence of elements in a structure's representative system, (see, for example, Zazworsky and Knudsen [103]) the theory relates to LTI state-

space I-structures. In essence, an I-structure is generically controllable if, for almost any parameter value, one can drive the state vector of the corresponding system into any desired state with a suitable choice of input.

This property is formally defined in Definition 3.27 for a continuous-time LTI state-space I-structure. This definition follows the presentation of Vajda *et al.* [71], which aimed to make the statement of the property more explicit than does Lin [47].

Definition 3.27. For an LTI state-space I-structure of n states and parameter set Θ, consider the matrix

$$\mathbf{Q}(\boldsymbol{\theta}) \triangleq \begin{bmatrix} \mathbf{B}(\boldsymbol{\theta}) & \mathbf{A}(\boldsymbol{\theta})\mathbf{B}(\boldsymbol{\theta}) & \cdots & \mathbf{A}(\boldsymbol{\theta})^{n-1}\mathbf{B}(\boldsymbol{\theta}) \end{bmatrix}. \tag{3.6.33}$$

The structure is **generically controllable** if for almost all $\boldsymbol{\theta} \in \Theta$

$$\text{rank } \mathbf{Q}(\boldsymbol{\theta}) = n. \tag{3.6.34}$$

Remark 3.13. While Definition 3.27 is intended for LTI I-structures, there are various occurrences in the literature (such as Vajda *et al.* [71] and Zhang *et al.* [104]) where the test for controllability is mentioned in association with compartmental structures. This seems at variance with the approach outlined for such structures in Remark 3.8.

Further, the state space condition of (3.6.34) seems inappropriate for a positive structure. To use the example of structures with a state vector of n elements, the maximal state space of a positive structure is $\bar{\mathbb{R}}^n_+$ whereas it is $\mathbb{R}^n$ for a LTI structure.

Definition 3.27 does not make any reference to the initial state of a system. In structures of systems where the initial state is unknown, as occurs in biological or chemical systems, it is appropriate to include the initial state in describing the properties of a structure. We address this in the generalisation of Definition 3.27 given below.

3.6.3.2 Generic weak reachability

The notion of generic weak reachability of a LTI structure given in van den Hof [81, 82] is adapted here so that the definition relates to LTI I-structures, removing one potential source of misinterpretation.

Definition 3.28. Consider an LTI state-space I-structure with parameter set Θ, as illustrated by

$$\begin{aligned} \dot{\mathbf{x}}(t,\boldsymbol{\theta}) &= \mathbf{A}(\boldsymbol{\theta})\mathbf{x}(t,\boldsymbol{\theta}) + \mathbf{B}(\boldsymbol{\theta})\mathbf{u}(t), \quad \mathbf{x}(0,\boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}), \\ \mathbf{y}(t,\boldsymbol{\theta}) &= \mathbf{C}(\boldsymbol{\theta})\mathbf{x}(t,\boldsymbol{\theta}) + \mathbf{D}(\boldsymbol{\theta})\mathbf{u}(t), \end{aligned} \tag{3.6.35}$$

and let $f : \Theta \to SL\Sigma P(n,m,k)$ be the parameterisation map such that for $\boldsymbol{\theta} \in \Theta$

$$f(\boldsymbol{\theta}) = (\mathbf{A}(\boldsymbol{\theta}), \mathbf{B}(\boldsymbol{\theta}), \mathbf{C}(\boldsymbol{\theta}), \mathbf{D}(\boldsymbol{\theta}), \mathbf{x_0}(\boldsymbol{\theta})).$$

Recalling $\mathbf{Q}$ from (3.6.33) and defining the matrices

$$\mathbf{P}(\boldsymbol{\theta}) = \begin{bmatrix} \mathbf{x_0}(\boldsymbol{\theta}) & \mathbf{A}(\boldsymbol{\theta})\mathbf{x_0}(\boldsymbol{\theta}) & \cdots & \mathbf{A}(\boldsymbol{\theta})^{n-1}\mathbf{x_0}(\boldsymbol{\theta}) \end{bmatrix}$$

and

$$\mathbf{R}(\boldsymbol{\theta}) = \begin{bmatrix} \mathbf{Q}(\boldsymbol{\theta}) & \mathbf{P}(\boldsymbol{\theta}) \end{bmatrix},$$

then the structure is **generically weakly reachable** if for almost all $\boldsymbol{\theta} \in \Theta$

$$\text{rank } \mathbf{R}(\boldsymbol{\theta}) = n.$$

In this case one says that $\Big(\mathbf{A}(\cdot), (\mathbf{B}(\cdot)\ \mathbf{x_0}(\cdot))\Big)$ is a generically reachable pair.

Remark 3.14. Definition 3.28 has its origins in van den Hof [81] which considers testing "structured linear systems" — including structures of positive and compartmental systems — for global *a priori* identifiability. Such systems may not have matrices that are structured (see Definition 3.13). Thus the concerns noted in Remark 3.13 may apply similarly to the testing of a compartmental LTI structure for generic weak reachability by Definition 3.28.

3.6.3.3 Generic observability

As for generic controllability, the property of generic observability is intended for a structure of systems having independent matrix elements (Zazworsky and Knudsen [103]), that is, an I-structure. An I-structure has the property of generic observability if, for systems in the structure corresponding to almost all feasible parameter values, it is possible to determine the underlying state of the system from the system output for all time. This property is more formally specified in Definition 3.29, which draws on the presentation of Vajda *et al.* [71].

Definition 3.29. An LTI state-space I-structure of n states and parameter set Θ is **generically observable** if, for almost all $\boldsymbol{\theta} \in \Theta$,

$$\operatorname{rank} \begin{bmatrix} \mathbf{C}(\boldsymbol{\theta}) \\ \mathbf{C}(\boldsymbol{\theta})\mathbf{A}(\boldsymbol{\theta}) \\ \vdots \\ \mathbf{C}(\boldsymbol{\theta})\mathbf{A}(\boldsymbol{\theta})^{n-1} \end{bmatrix} = n.$$

In this case one says that $(\mathbf{A}(\cdot), \mathbf{C}(\cdot))$ is a generically observable pair.

The usage of Definition 3.29 in the classification of compartmental LTI structures may be inappropriate for the reasons noted in Remark 3.13.

3.6.3.4 Generic minimality

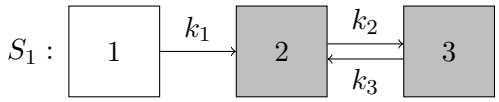
In considering LTI structures, van den Hof [81] classifies a compartmental structure as generically (structurally) minimal (recall Definition 3.20) if and only if it is generically weakly reachable (Definition 3.28) and generically observable (Definition 3.29). Earlier studies, such as Vajda *et al.* [71], infer generic minimality of a compartmental structure from generic observability and generic controllability (Definition 3.27), even though the initial conditions may be nonzero. Ascribing minimality to a system by virtue of its observability and controllability is due to Kalman [39].

As noted in Whyte [90,91] and Remark 3.13, it is not appropriate to apply these definitions to a structure that is not an I-structure. Hence, the use of certain generic properties to infer generic minimality of a compartmental structure also seems inappropriate. As a result, we require an alternative means of testing the compartmental structures we consider in this thesis for generic minimality.

Gilbert [89] considered the matter of when the impulse response function of a controlled state-space LTI system of n compartments is a sum of fewer than n exponential terms. This is suggestive of a structure which is not minimal. The paper has some inaccuracies, some of which may be typographical errors, and some which may have a more significant impact on the arguments presented. Regardless, there are certain restrictions on the systems under consideration, most notably the assumption that the initial state is a zero vector. Also, systems considered are examined in a manner which is not so closely related to more modern treatments where a structure is tested for the generic presence of a property in an explicit sense. In particular, the examples presented use $\mathbf{A}$ matrices with purely numerical values, rather than either parameters or zero elements. It is possible that some of the arguments of Gilbert [89] may be adapted for the LTI state-space structures we consider in this thesis, in which $\mathbf{x_0}$ and $\mathbf{C}$ contain parameters.

One approach referenced in Gilbert [89] and elsewhere to judging whether or not an LTI state-space structure is generically minimal requires inspection of the Laplace transform of response of a structure's representative system. This process is applicable regardless of whether or not the structure is positive. Further, this process is an implicit part of (at least) one method of testing a structure for global *a priori* identifiability. Our examination of this practice leads to its modification for use with the ULSS-1 structures we consider later.

To illustrate the importance of establishing whether or not a structure has the property of generic minimality, let us consider a toy example. We note that this example is obviously not generically minimal, yet it is useful as it allows us to demonstrate the concept without the distraction of undue algebraic complexity.



$$\frac{d}{dt}\begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = \begin{bmatrix} -k_1 & 0 & 0 \\ k_1 & -k_2 & k_3 \\ 0 & k_2 & -k_3 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix}$$

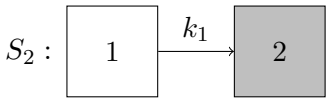
$$y_1(t) = \begin{bmatrix} 0 & 1 & 1 \end{bmatrix} \mathbf{x}(t)$$

$$\mathbf{x}(0) = \begin{bmatrix} a & 0 & 0 \end{bmatrix}^\top \in \mathbb{R}^3_+$$

$$\boldsymbol{\rho} = (k_1,\, k_2,\, k_3,\, a)^\top \in \mathbb{R}^4_+$$

$$\mathcal{L}\{y_1\}(s, \boldsymbol{\rho}) = \frac{ak_1 \cdot \cancel{(s + k_2 + k_3)}}{s(s + k_1)\cancel{(s + k_2 + k_3)}}$$

(a) Diagram for and specification of a three-compartment uncontrolled structure.



$$\frac{d}{dt}\begin{bmatrix} z_1 \\ z_2 \end{bmatrix} = \begin{bmatrix} -k_1 & 0 \\ k_1 & 0 \end{bmatrix} \begin{bmatrix} z_1 \\ z_2 \end{bmatrix}$$

$$y_2(t) = \begin{bmatrix} 0 & 1 \end{bmatrix} \mathbf{z}(t)$$

$$\mathbf{z}(0) = \begin{bmatrix} a & 0 \end{bmatrix}^\top \in \mathbb{R}^2_+$$

$$\boldsymbol{\pi} = (k_1,\, a)^\top \in \mathbb{R}^2_+$$

$$\mathcal{L}\{y_2\}(s, \boldsymbol{\pi}) = \frac{ak_1}{s(s + k_1)}$$

(b) Diagram for and specification of a two-compartment uncontrolled structure.

Figure 3.6.2: A three-compartment model structure S_1 (left) is not generically minimal as it is generically indistinguishable from a two-compartment structure S_2 (right). Shaded compartments indicate those which contribute to a structure's response function.

Consider the two uncontrolled state-space (compartmental) LTI structures presented in Figure 3.6.2. Both are defined for time set $T = \bar{\mathbb{R}}_+$. Figure 3.6.2a shows a compartmental diagram for the representative system of a three-compartment structure S_1, with the joint observation of compartments labelled 2 and 3 providing the output. The representative system of S_1 (say $S_1(\boldsymbol{\rho})$) is provided beneath the compartmental diagram. Figure 3.6.2b similarly presents a diagram of the two-compartment structure S_2 and its representative system (say $S_2(\boldsymbol{\pi})$). The output of S_2 is due to observation of its compartment 2.

Let us consider the Laplace transform of the output of these representative systems. By Remark 3.9, each Laplace transform exists on some domain.

Let the Laplace transform of the response of $S_1(\boldsymbol{\rho})$ and $S_2(\boldsymbol{\pi})$ be $\mathcal{L}\{y_1\}(s, \boldsymbol{\rho})$ as shown in Figure 3.6.2a, and $\mathcal{L}\{y_2\}(s, \boldsymbol{\pi})$ as shown in Figure 3.6.2b, respectively. By deriving the rational function for $\mathcal{L}\{y_1\}(s, \boldsymbol{\rho})$ and factorising its numerator and denominator, we see that pole-zero cancellation can occur for any feasible $\boldsymbol{\rho}$. We note that pole-zero cancellation is not possible in $\mathcal{L}\{y_2\}(s, \boldsymbol{\pi})$, and hence S_2 is generically minimal.

By cancelling the common factor in $\mathcal{L}\{y_1\}(s, \boldsymbol{\rho})$, we see that its canonical form is equivalent to that of $\mathcal{L}\{y_2\}(s, \boldsymbol{\pi})$. Hence, we can reduce a system in Figure 3.6.2a to one in Figure 3.6.2b for any feasible $\boldsymbol{\rho}$, which shows that S_1 is not generically minimal. Further, regardless of the number of observations made of a system in S_1 or the true value of the parameters, it is not possible to estimate the parameters k_2 and k_3 from data. As a result of this structural feature, S_1 is *a priori* unidentifiable by Definition 3.17.

This example demonstrates that a non-canonical form of the Laplace transform of a representative system's response may overstate the amount of parameter information actually present in system response. Using such erroneous information in a test of a structure for global *a priori* identifiability may cause us to classify a structure incorrectly. The possibility of such an undesirable result emphasizes a vital step in the Laplace transform method — the necessity of completing any possible pole-zero cancellation before proceeding with the test.

We will formalise our comments on defining generic minimality for an uncontrolled LTI state-space structure in the following definition.

Definition 3.30. For some uncontrolled LTI state-space structure M of n state variables, let n be the 'apparent number of state variables', which is the degree of the denominator in the unprocessed $\mathcal{L}\{y\}(s)$ of Equation (3.6.31). The canonical form of $\mathcal{L}\{y\}(s)$ presented in (3.6.32) shows that r is the 'effective number of state variables' of M. If $r = n$ then M is generically minimal. If $r < n$ then M is not generically minimal as M is generically indistinguishable from a structure of $r < n$ state variables.

We are now able to employ the definitions of this chapter in describing how to test an LTI structure (compartmental or otherwise) for global *a priori* identifiability. We will illustrate this process with an example. Aspects of this treatment will inform our consideration of compartmental ULSS structures in Chapter 4.

3.6.4 Testing uncontrolled LTI structures for global *a priori* identifiability

Bellman and Åström [6] proposed the initial approach to testing a LTI state-space structure for global *a priori* identifiability. They considered a compartmental structure which arose from the modelling of a biological system. Their testing approach used the Laplace transform of system response. The process determined and utilized the response invariants of a generically minimal structure obtainable from the original, without explicitly determining this minimal structure. These invariants ($\phi_i(\boldsymbol{\theta}) \not\equiv 0$, $i = 0, \ldots, r+p$ as in Equation (3.6.32)) were as required by Definition 3.19. Bellman and Åström's process was used in the testing of compartmental LTI structures by other authors, see, for example, Vajda and Rabitz [80].

There are various other methods of testing a LTI structure for global *a priori* identifiability, such as the similarity transform approach [30] and the Markov parameter approach. The tests are distinguished by the manner in which they define the invariants of the structure — recall Section 3.6.2.4 for examples. Unlike the Laplace transform approach, these alternative tests are only applicable to a generically minimal structure. As a result, they require a preliminary step of reducing a structure which is not generically minimal to one that has this property.[15]

The ability of the Laplace transform method to test an LTI structure which is not generically minimal for global *a priori* identifiability gives it an advantage over other methods. This feature is particularly useful in our the analysis of subsystems of ULSS that we present in Chapter 4. As a result, we present the use of the Laplace transform

[15] Recall the discussion of the importance of generic minimality to the testing of a structure for global *a priori* identifiability attending Figure 3.6.2.

method in detail below.

Remark 3.15. Many of the tests of a structure for global *a priori* identifiability do not readily allow us to apply our results to a related structure. That is, we must perform a separate analysis of the second structure. Yates [102] considered this issue in the context of a certain type of LTI structures termed "Physiologically Based Pharmacokinetic Models" (PBKMs). We may think of these structures as extensions of the "mamillary" class of compartmental structures. (We showed an example of these structures in Figure 1.1.2. The characteristic feature is a central compartment which exchanges mass with other (peripheral) compartments. There is not any direct flow of mass between peripheral compartments.)

Experimentalists may attempt to use PBKMs to ascertain the amount of a drug over time in living tissue. As in our application, they may intend to estimate parameters such as rate constants from data. An experimentalist may modify an initial structure to include more detail on how a drug moves between bodily compartments or is eliminated to the environment. Most simply, they may achieve this by replacing some peripheral compartment with a subsystem of two or more compartments. Yates [102] considered an initial PBKM and structures obtained from it by some modification. The paper demonstrated that some of the results obtained from testing the original structure for global *a priori* identifiability were transferable to the consideration of a modified structure. We will consider whether we can profitably extend this approach to LSS structures in future work.

3.6.4.1 A simple example

By considering an uncontrolled LTI state-space model structure, we may illustrate the process of testing a structure for global *a priori* identifiability without requiring the complex method needed for the LSS structures to come. For a relatively simple example, recall

the chemical reaction system (2.2.9):

$$\begin{array}{ccccc} \mathrm{X} & \xrightarrow{k_3} & \mathrm{R} & \xrightarrow{k_7} & \mathrm{D_R} \\ \mathrm{Y} & \underset{k_4}{\longrightarrow} & \mathrm{S} & \underset{k_8}{\longrightarrow} & \mathrm{D_S} \end{array} \quad . \tag{2.2.9}$$

Reformulating the rate equations given for (2.2.9), that is, (2.2.12) and (2.2.13), as a state-space system in terms of a state vector $\mathbf{x}$, response $\mathbf{y}$, and unspecified parameter $\boldsymbol{\theta}$ gives:

$$\begin{gathered} \dot{\mathbf{x}}(t,\boldsymbol{\theta}) = \mathbf{A}(\boldsymbol{\theta})\mathbf{x}(t,\boldsymbol{\theta}), \quad \mathbf{x}(0,\boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}), \\ \mathbf{y}(t,\boldsymbol{\theta}) = \mathbf{C}\mathbf{x}(t,\boldsymbol{\theta}), \end{gathered} \tag{3.6.36}$$

where $t \in \bar{\mathbb{R}}_+$ and

$$\begin{gathered} \mathbf{x}(t,\boldsymbol{\theta}) = \begin{bmatrix} \mathrm{X}(t,\boldsymbol{\theta}) \\ \mathrm{Y}(t,\boldsymbol{\theta}) \\ \mathrm{R}(t,\boldsymbol{\theta}) \\ \mathrm{S}(t,\boldsymbol{\theta}) \end{bmatrix}, \quad \mathbf{A}(\boldsymbol{\theta}) = \begin{bmatrix} -k_3 & 0 & 0 & 0 \\ 0 & -k_4 & 0 & 0 \\ k_3 & 0 & -k_7 & 0 \\ 0 & k_4 & 0 & -k_8 \end{bmatrix}, \quad \mathbf{x_0}(\boldsymbol{\theta}) = \begin{bmatrix} X_0 \\ Y_0 \\ 0 \\ 0 \end{bmatrix}, \\ \mathbf{C} = \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad \text{and} \quad \boldsymbol{\theta} = (X_0, Y_0, k_3, k_4, k_7, k_8)^\top \in \mathbb{R}^6_+. \end{gathered} \tag{3.6.37}$$

We use (3.6.36) and (3.6.37) to define the representative system of structure $\mathcal{M}_0$, an uncontrolled (and compartmental) LTI state-space structure.

In order to test $\mathcal{M}_0$ for global *a priori* identifiability as described in Section 3.6.4, let us employ the Laplace transform method. We present details of the calculations and some of our subroutines that assist with these in the Maple worksheet of Appendix B. (Some of these routines also feature in our consideration of ULSS structure test cases in Chapter 5.) The test requires the moment invariants from the Laplace transform of the response of (3.6.36) subject to (3.6.37). Adapting the expression given for $\mathcal{L}\{y(\cdot,\boldsymbol{\theta})\}$ in (3.6.32) for the case of a vector response gives

$$\mathcal{L}\{y(\cdot,\boldsymbol{\theta})\}(s) = \begin{bmatrix} \dfrac{k_3 X_0}{s^2 + (k_3+k_7)s + k_3 k_7} \\ \dfrac{k_4 Y_0}{s^2 + (k_4+k_8)s + k_4 k_8} \end{bmatrix}. \tag{3.6.38}$$

Each component of (3.6.38) is in the canonical form (Definition 3.26) as factor cancellation is clearly not possible in either case. Thus, the collection of coefficients of s in (3.6.38) provides a vector of moment invariants $\boldsymbol{\phi}(\boldsymbol{\theta})$. Hence, defining

$$\boldsymbol{\phi}(\boldsymbol{\theta}) = \Big(k_3 X_0, \quad k_3 + k_7, \quad k_3 k_7, \quad k_4 Y_0, \quad k_4 + k_8, \quad k_4 k_8 \Big)$$

allows us to form the equations $\boldsymbol{\phi}(\boldsymbol{\theta}') = \boldsymbol{\phi}(\boldsymbol{\theta})$ required by the *a priori* identifiability test described by (3.5.20).

These equations have feasible solutions:

$$\mathcal{I}(\mathcal{M}_0, \boldsymbol{\theta}) = \left\{ \boldsymbol{\theta}' \in \mathbb{R}^6_+ \;\middle|\; \begin{array}{l} \{X_0' = X_0, \quad k_3' = k_3,\ k_7' = k_7,\ Y_0' = Y_0, \quad k_4' = k_4,\ k_8' = k_8\}, \\ \{X_0' = X_0, \quad k_3' = k_3,\ k_7' = k_7,\ Y_0' = \dfrac{Y_0 k_4}{k_8},\ k_4' = k_8,\ k_8' = k_4\}, \\ \{X_0' = \dfrac{X_0 k_3}{k_7},\ k_3' = k_7,\ k_7' = k_3,\ Y_0' = Y_0, \quad k_4' = k_4,\ k_8' = k_8\}, \\ \{X_0' = \dfrac{X_0 k_3}{k_7},\ k_3' = k_7,\ k_7' = k_3,\ Y_0' = \dfrac{Y_0 k_4}{k_8},\ k_4' = k_8,\ k_8' = k_4\} \end{array} \right\}, \quad (3.6.39)$$

from which we classify structure $\mathcal{M}_0$ according to Definition 3.17.

As (3.6.39) shows a finite number of solutions that is greater than one for any $\boldsymbol{\theta} \in \mathbb{R}^6_+$, we classify $\mathcal{M}_0$ as locally *a priori* identifiable. Inspection of (3.6.39) reveals the source of multiple solutions in $\mathcal{I}(\mathcal{M}_0, \boldsymbol{\theta})$. The first solution, $\boldsymbol{\theta}' = \boldsymbol{\theta}$, is present in any test of a structure for global *a priori* identifiability as it is trivially true. The second solution shows that for the reaction pathway involving Y in (2.2.9), given the response of the system having the parameter vector $\boldsymbol{\theta}$, the time course of S is unchanged if we interchange the values of k_4 and k_8 and scale the initial conditions. The third solution shows that analogous changes to parameters in the pathway involving X do not change the time course of R. The fourth solution follows from the previous two and the independence of the two reactions.

Some structures — such as those we will employ to describe flow-cell optical biosensor experiments — are subject to exogenous variables. It is useful to appreciate the limitations of what we can discern from testing such a structure for global *a priori* identifiability.

This requires us to consider the underpinnings of such a test. We will consider this for LTI structures, although the reasoning behind our comments applies to other classes of structures.

3.6.4.2 Global *a priori* identifiability for an LTI structure subject to experimental variables

The framework used for testing a structure for global *a priori* identifiability states certain assumptions (outlined in Section 1.3), including that the structure is the correct representation of the physical system. In effect, this is used as a shorthand for another assumption.

Assumption 3.1. Observations of some physical system are suitably represented by the output of a model structure having r effective state variables (recall Definition 3.30) where this output is subject to random noise that is independent of parameter values. That is, the idealised data obtained from the structure will encapsulate the features of the deterministic part of experimental data. As such, it is appropriate to use this idealised data in testing the structure for global *a priori* identifiability.

Assumption 3.1 may not be reasonable when we consider structures that also depend on experimental (or otherwise exogenous) variables. (Most simply, these may occur in a system's initial conditions or the system matrices of a linear system.) For example, suppose that Assumption 3.1 is true for some combinations of experimental variables and parameter values, yet for others, data is appropriately modelled by a structure of $n_1 < r$ state variables. We will refer to such an event as a **structure-data mismatch**.

An *a priori* identifiability analysis which does not recognise a structure-data mismatch may employ an inappropriate idealisation of the intended parameter estimation problem. As such, the result obtained from the analysis may not accurately judge the value of the planned experiments. Hence, it is desirable to know of any conditions for

which Assumption 3.1 is violated prior to testing a structure for global *a priori* identifiability. We may use this information in the planning of experiments to avoid a structure-data mismatch.

To the best of our knowledge, the analysis of a structure in the literature implicitly assumes that Assumption 3.1 holds. This may be a consequence of the usual textbook considerations of *a priori* identifiability analysis employing linear structures with system matrices that are not subject to exogenous variables.[16] We will explore a particular situation in which Assumption 3.1 is invalid.

Denis-Vidal and Joly-Blanchard [22] considered the mismatch of experimental and idealised data in a limited sense. They proposed conditions on a structure which ensured that idealised data obtained from it would avoid one situation that violated Assumption 3.1. Only a structure that satisfied their conditions would proceed to testing for global *a priori* identifiability. We commented on the conditions of Denis-Vidal and Joly-Blanchard [22] in Remark 3.4.[17] We will illustrate their relevance to Assumption 3.1 with an example.

Example 3.1. Consider an uncontrolled compartmental LTI structure M (as in Definition 3.24) of n states with scalar output y, defined for $T = \bar{\mathbb{R}}_+$. Ordinarily, we would expect that the idealised response of M's representative system allows us to obtain a set of invariants, as in (3.6.32). These determine the set of parameter values able to produce the idealised response. However, suppose this representative system has an initial state $\mathbf{x}(0) = \mathbf{x}^*$, which is an equilibrium state. As a result, the system's initial response, $y(0) = \mathbf{c}\mathbf{x}^* \triangleq y^*$, is the response for all time. Hence

$$\mathcal{L}\{y\}(s) = \frac{y^*}{s} \quad \text{for } s \in \mathrm{H}_0\,, \tag{3.6.40}$$

and this is in the required canonical form (recall Definition 3.26). If $y^* > 0$, then (3.6.40)

[16] Two examples of where this is the case for compartmental models are Godfrey [29, Chapter 6], and Seber and Wild [73, Chapter 8].

[17] We will include conditions inspired by [22] in the consideration of ULSS-1 structures we will present in Definition 4.1.

gives one invariant. If $y^* = 0$, then (3.6.40) does not provide any parameter information.

We see that the idealised data from this example would violate Assumption 3.1 if we expected $r \geq 1$. To appreciate the importance of this, suppose that we use an expression such as (3.6.40) in classifying M. It is possible that using idealised data which satisfies Assumption 3.1 to classify M will give a different result. This is because we expect that an expression such as (3.6.32) will give us a greater number of invariants to use in a test than the single one obtainable from (3.6.40).

Using (3.6.40) in an *a priori* identifiability analysis of some structure is only valid if we expect that the experimental data we collect will be constant for all time. We expect such data to be less useful for parameter estimation than a time-varying output. As such, we would seek to choose our conditions to avoid such 'degenerate' experiments.

This discussion shows that awareness of whether Assumption 3.1 is valid or not is useful in judging if the test of an assumed structure for global *a priori* identifiability is a meaningful exercise.

We will consider other situations in which Assumption 3.1 is invalid when we consider the particulars of our test case structures in Chapter 5.

In the next section we define a structure of uncontrolled linear switching systems (ULSSs). These are suitable for modelling the observations obtained from kinetic experiments of two phases (as described in Section 2.4.2 and Definition 2.3) performed under certain conditions.

3.7 Structures of uncontrolled linear switching systems (ULSSs)

We are interested in physical systems that are appropriately modelled by a class of uncontrolled linear switching system structures. The basic unit of these — a linear switching

system[18] defined for time set T — has two key elements. The first is a collection of LTI state-space systems. The second is a means of determining which of these systems is in effect at any given time.

Representations of system classes in the control theory literature may eschew formal definitions at times, preferring to illustrate key features of the system. This is the case in Sun and Ge [77], which considers discrete-time nonlinear switching systems and linear switching systems subject to disturbances. Further, the switching systems may have a switching device termed the **supervisor** which sets the switching rule γ. In general, γ may depend on some combination of the time, previous values of γ, the state, the output or an external signal. While Sun and Ge [77] is comprehensive, the complexity of the switched systems and their general switching rules presented there is not necessary for the applications we consider in this thesis.

Instead, we draw inspiration from Ezzine and Haddad's presentation of discrete-time uncontrolled linear switching systems [27]. They assumed a simplified form of switching function that is suitable for our application. Using Ezzine and Haddad [27] as a starting point, we propose a definition of a structure of continuous-time uncontrolled linear switching systems (an ULSS structure) that is appropriate for our purposes. Shortly we will formalise some details of the ULSS structure's constituent systems. We will also present a class of ULSS structures that is of particular use to us in this thesis.

Definition 3.31. For some time interval T, define the deterministic **switching function** $\gamma(\cdot)$ by $\gamma : T \to \Gamma : t \mapsto \gamma(t) \in \Gamma$, $\Gamma \subset \mathbb{N}$.

Consider $\Gamma = \{1, \ldots, q\} \subset \mathbb{N}$ for $q > 1$. Further, consider unspecified parameter vectors $\boldsymbol{\theta}_i \in \Theta_i$, $i = 1, \ldots, q$, combined (recall (3.1.1)) to give $\boldsymbol{\theta} \triangleq \langle \boldsymbol{\theta}_1, \ldots, \boldsymbol{\theta}_q \rangle$. An **uncontrolled linear switching system structure of q component systems** defined for time interval T is a collection of state-space systems for which state variables $\mathbf{x}$ and outputs $\mathbf{y}$

[18] These are also known by terms which include switched, jump, and hybrid systems.

at time $t \in T$ are related as shown in the representative system

$$\begin{aligned} \dot{\mathbf{x}}(t, \boldsymbol{\theta}) &= \mathbf{A}_{\gamma(t)}(\boldsymbol{\theta}_{\gamma(t)})\mathbf{x}(t, \boldsymbol{\theta}), \quad \mathbf{x}(0, \boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}_1), \\ \mathbf{y}(t, \boldsymbol{\theta}) &= \mathbf{C}_{\gamma(t)}(\boldsymbol{\theta}_{\gamma(t)})\mathbf{x}(t, \boldsymbol{\theta}). \end{aligned} \tag{3.7.41}$$

The value of γ at any time dictates which of the q component uncontrolled LTI state-space systems is in effect at that time. The i-th ($i \in \Gamma$) component system has the form

$$\begin{aligned} \dot{\mathbf{x}}(t, \boldsymbol{\theta}) &= \mathbf{A}_i(\boldsymbol{\theta}_i)\mathbf{x}(t, \boldsymbol{\theta}), \\ \mathbf{y}(t, \boldsymbol{\theta}) &= \mathbf{C}_i(\boldsymbol{\theta}_i)\mathbf{x}(t, \boldsymbol{\theta}). \end{aligned} \tag{3.7.42}$$

Suppose the state and output spaces are $X = \mathbb{R}^n$ and $Y = \mathbb{R}^k$ respectively (recall Definition 3.8). Then for $i \in \Gamma$, $\mathbf{A}_i \in \mathbb{R}^{n \times n}$ and $\mathbf{C}_i \in \mathbb{R}^{k \times n}$. The structure's representative system (3.7.41) has the property that all matrices have elements that are either zero or an expression involving the parameters.

An ULSS structure is characterized by the pattern of non-zero elements appearing in $\mathbf{x_0}$ (as in (3.7.41)) and each of the $\mathbf{A}_i$ and $\mathbf{C}_i$ for $i = 1, \ldots, q$ that appear in a representative system of an LTI structure, as in (3.7.42).

Remark 3.16. A switching system may also be known as a **switched autonomous system** or an **unforced switching system**. A component system of a switching system may also be known as a **subsystem** or **mode** of the switched system.

Our application directs us towards particular interest in a special case of Definition 3.31. In particular, we consider ULSS structures for which the switching function has one switching event. Henceforth we will term these ULSS-1 structures.

Definition 3.32. An **ULSS-1 structure** is a type of uncontrolled linear switching system structure as in Definition 3.31 which has two component systems (that is, $q = 2$). Further, the switching function $\gamma(\cdot)$ has $\Gamma = \{1, 2\}$ and is defined by

$$\gamma(t) = \begin{cases} 1, & 0 \leq t < t_1, \\ 2, & t \geq t_1, \end{cases} \tag{3.7.43}$$

where $t_1 > 0$ is the **switching time**. We assume that $\mathbf{x}(t_1, \boldsymbol{\theta}) = \mathbf{x}(t_1^-, \boldsymbol{\theta})$.

An ULSS-1 structure is characterized by the pattern of non-zero elements of $\mathbf{x_0}$ (as it appears in an expression of the form of (3.7.41)) and $\mathbf{A}_i$ and $\mathbf{C}_i$ for $i = 1, 2$ that appear in LTI representative systems (as in (3.7.42)).

Definition 3.33 (Parameters in an ULSS-1 structure)**.** Suppose an ULSS-1 structure has feasible parameter set Θ, and $\boldsymbol{\theta} \in \Theta$ is an unspecified vector of parameters that serves to illustrate the structure via a representative system. As a result of this choice, we can consider the ULSS-1 representative system to be composed of LTI subsystems, each of which is representative of some LTI structure. Given a particular ULSS-1 representative system, we can use the LTI system in effect prior to the switching event to define what we will call the "phase 1" LTI structure. Similarly, the LTI system in effect after the switching event defines the "phase 2" LTI structure.

There are situations in which some parameters present in the phase 1 structure are absent from the phase 2 structure, and *vice versa*. As such, we will find it useful to employ notation that can distinguish between the parameters that occur in these two phases.

For $i = 1, 2$, we define $\boldsymbol{\theta}_i$ as the vector of distinct parameters which appear in the phase i structure's system matrices (and the initial condition vector for $i = 1$). For $i = 1, 2$ we choose Θ_i such that $\boldsymbol{\theta}_i \in \Theta_i$. In particular we require Θ_i that is an appropriate parameter space given the number of parameters and any *a priori* information on their feasible values.

In order to relate ULSS-1 structures to the literature we reviewed earlier, we may define an ULSS-1 I-structure as a special case of the structured linear time-varying systems presented in Definition 3.16.

Definition 3.34. An ULSS-1 structure (Definition 3.32) is a **ULSS-1 I-structure** if

- $\mathbf{A_2}$ has the same pattern as $\mathbf{A_1}$ and $\mathbf{C_2}$ has the same pattern as $\mathbf{C_1}$,

- the collection of all nonzero entries of the matrices $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{C_1}$, $\mathbf{C_2}$ and $\mathbf{x}_0$ is algebraically independent.

3.8 Prelude to Chapter 4

In the following chapter, we propose a definition of global *a priori* identifiability for an ULSS-1 structure that is based on that of Whyte [90, 91]. Our definition is inspired by features of the state-space LTI structures discussed in this chapter.

We then turn our attention to the process of testing an ULSS-1 structure for the property of global *a priori* identifiability. We note difficulties with the theory originally proposed for this purpose in [90,91]. We recapitulate a method (first presented in Whyte [94]) aimed at inferring that an ULSS-1 structure is globally *a priori* identifiable. We do this because of the method's success in circumventing certain difficulties in the original testing process. This success led to the classification of a previously unclassified structure drawn from the flow-cell optical biosensor literature. The method was shown to be a sufficient means of classifying a particular ULSS-1 structure as globally *a priori* identifiable. In certain cases, the method may also provide a check on the classification of a structure obtained by other methods. However, this indirect method does not directly address the limitations of the original theory. Hence, in the remainder of the chapter we propose extensions to the testing process such that it can overcome difficulties inherent in Whyte [90, 91].

Chapter 4

Global *a priori* identifiability for structures of uncontrolled linear switching systems of one switching event

4.1 Overview

Recall the review of the literature on testing a structure of linear switching systems for global *a priori* identifiability given in Section 1.4. There we found that structures of continuous-time LSS had received little attention aside from in Whyte [90, 91]. These papers were motivated by structures employed in the modelling of biomolecular interactions studied via flow-cell biosensor experiments. In particular, they considered the modelling of experiments in which the "simple bimolecular interaction" was assumed to occur.[1] As a result of the motivating application, the papers analysed a structure of continuous-time uncontrolled linear switching systems of one switching event (ULSS-1). The ULSS in the

[1] A ULSS-1 structure to represent this experimental system was presented in Whyte [99].

structure were compartmental.

In this chapter we refine and extend the theory of global *a priori* identifiability of ULSS-1 structures proposed in Whyte [90,91], following on from our outline in Section 3.7. We begin by presenting methods (or some discussion of them) that draws on material first presented in the aforementioned papers. As these are the only works of particular relevance to the problem at hand, we may not reiterate citations in the interests of brevity.

Our discussion of global *a priori* identifiability for a ULSS-1 structure requires a consideration of the relevance of the general definition of global *a priori* identifiability (Definition 3.17) to such a structure.

To give a précis of the approach we will use to analyse a ULSS-1 structure, recall that testing a structure for global *a priori* identifiability occurs in an idealised framework where we assume that an error-free and infinite output is obtainable from systems comprising the structure. Consider a ULSS-1 structure M with feasible parameter set Θ where for an unspecified $\boldsymbol{\theta} \in \Theta$, $M(\boldsymbol{\theta})$ is representative of systems in M. Suppose M models the output observed for a flow-cell optical biosensor experiment of two phases under a given biomolecular interaction. We present a schematic representation of the output of $M(\boldsymbol{\theta})$ in Figure 4.1.1, which shows the typical shape of a response curve. For simplicity, we assume that all parameters are introduced in the first subsystem of $M(\boldsymbol{\theta})$. We will relax this requirement later in this chapter.

We approach the problem of testing some ULSS-1 structure M for global *a priori* identifiability by using M to define two uncontrolled LTI structures; $M^{[1]}$ ($M^{[2]}$) representing the collection of systems in effect before (after) the switching event. That is, the first (second) subsystem of the representative system $M(\boldsymbol{\theta})$ defines the collection of uncontrolled LTI systems which constitute structure $M^{[1]}$ ($M^{[2]}$). We define both $M^{[1]}$ and $M^{[2]}$ for time set $T = \bar{\mathbb{R}}_+$. We transpose the original problem of obtaining parameter information from the idealised output of M to a consideration of the idealised outputs of $M^{[1]}$ and $M^{[2]}$. In Figure 4.1.2 we show the idealised (error-free, infinite output) response curves from $M^{[1]}(\boldsymbol{\theta})$ and $M^{[2]}(\boldsymbol{\theta})$ created from the idealised response of $M(\boldsymbol{\theta})$ as shown

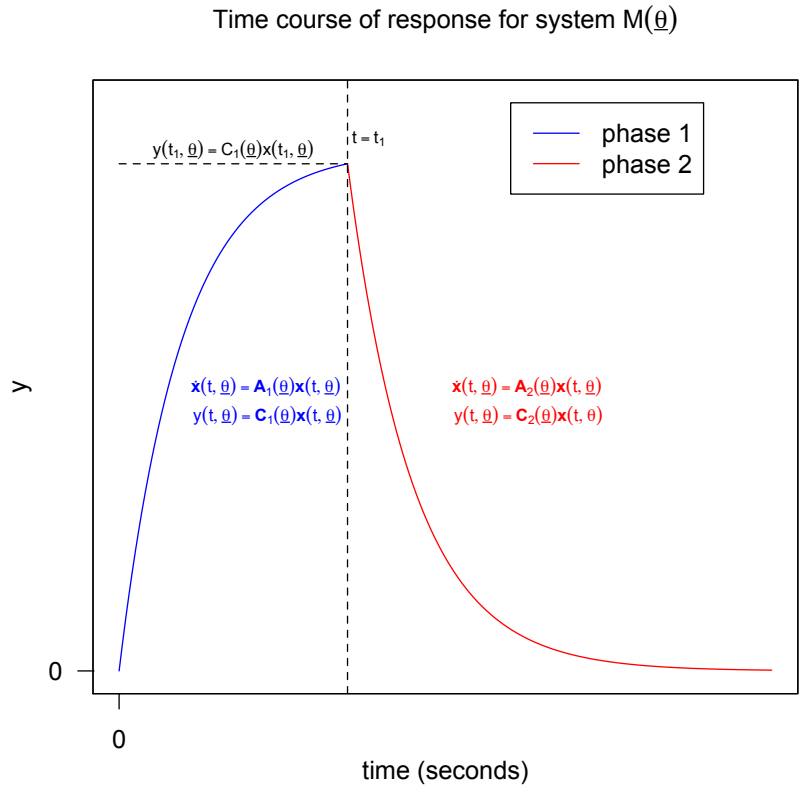


Figure 4.1.1: A schematic representation of the idealised response obtained from a scalar output ULSS-1 showing the subsystem in effect and the output it produces for the time prior to (after) the switching event in blue (red).

in Figure 4.1.1. In flow-cell optical biosensor experiments, the switching time t_1 is set by the experimentalist. As a result, the LTI subsystem $M^{[2]}(\boldsymbol{\theta})$ is constrained such that its initial state is determined by the state reached by $M^{[1]}(\boldsymbol{\theta})$ at time t_1.

The appeal of our proposed approach is that there are well-established techniques for obtaining parameter information from LTI structures, as seen in the literature on testing them for global *a priori* identifiability.[2] However, as the LTI systems in $M^{[1]}$ and $M^{[2]}$ are obtained from the ULSS-1 M, a system in $M^{[2]}$ depends on one from $M^{[1]}$ through

[2]Recall Section 3.6.4.

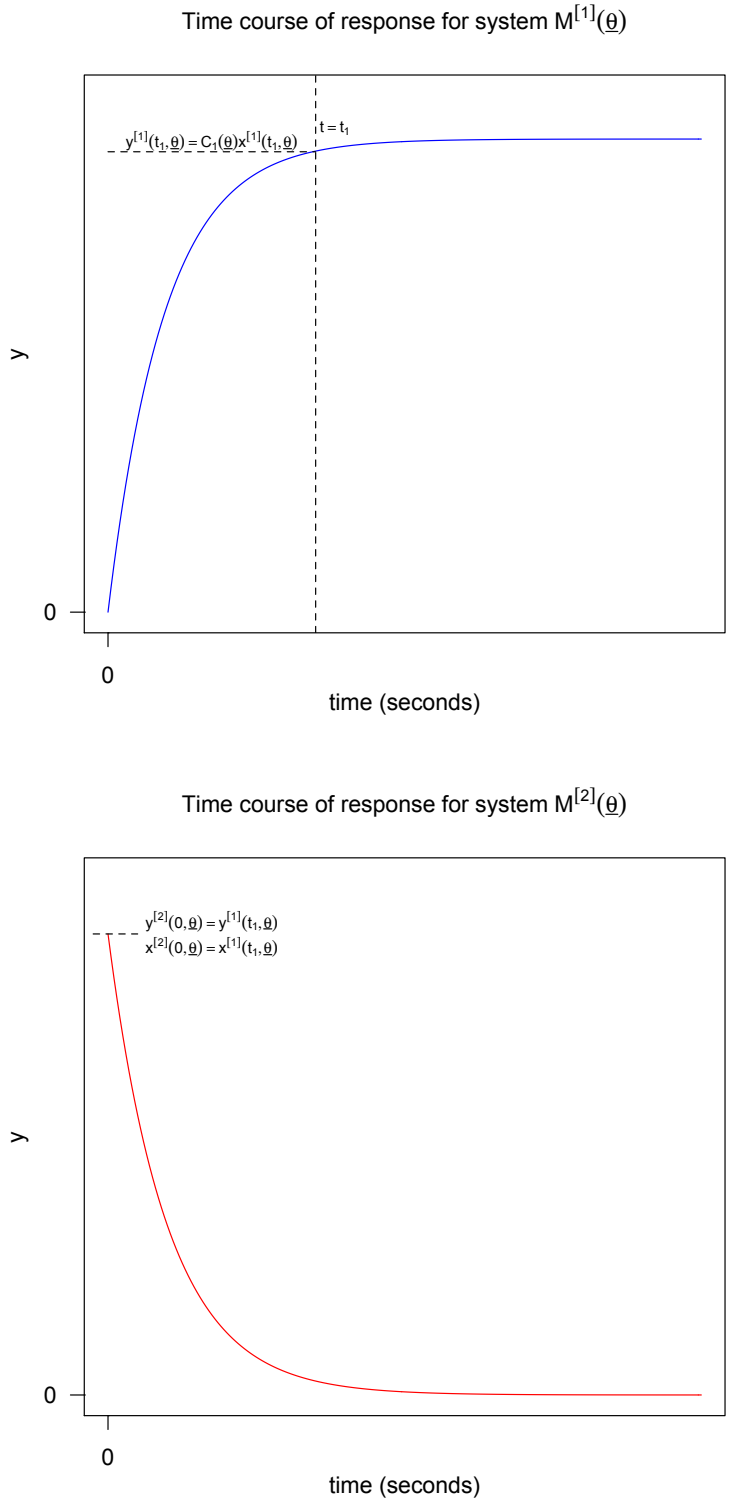


Figure 4.1.2: The idealised scalar output obtained from the LTI systems $M^{[1]}(\boldsymbol{\theta})$ (top) and $M^{[2]}(\boldsymbol{\theta})$ (bottom) derived from ULSS-1 $M(\boldsymbol{\theta})$ (response given in Figure 4.1.1).

the state of M. This presents some sub-problems that we must address in order to obtain information from the output of M[2]. We circumvent this difficulty through a method that is sufficient to infer global *a priori* identifiability. The method is able to obtain a definite result for a test case in Chapter 5. We follow this indirect approach with a more direct and more general method, which we also apply to test cases in Chapter 5.

4.2 A definition of global *a priori* identifiability for a ULSS-1 structure

A standard definition of global *a priori* identifiability for a structure (as we presented in Definition 3.17) assumes that the equations governing any constituent system do not change over time. With the exception of some degenerate systems, LSSs do not satisfy this condition. In general, any system in a ULSS-1 structure (as in Definition 3.32) on time set $T = [0, t_f)$ (where $t_f \in \mathbb{R}_+$) is governed by a different system on each of the time domains $t \in [0, t_1)$ and $t \in [t_1, t_f)$, where t_1 is the switching time of the ULSS-1 and $0 < t_1 < t_f$. It is appropriate to consider whether the conditions on the state evolution map $\mathbf{f}$ and the output map $\mathbf{h}$ suggested by Definition 3.17 are directly generalizable to a ULSS-1 structure, and if not, which other conditions are appropriate. To simplify this problem, let us utilise the LTI behaviour of a ULSS-1 on the time intervals $[0, t_1)$ and $[t_1, t_f)$.

Following (3.6.21), consider the representative system of an uncontrolled LTI state-space system structure defined for time set $T = [0, t_1)$:

$$\begin{gathered}\dot{\mathbf{x}}(t) = \mathbf{A}(\boldsymbol{\theta})\mathbf{x}(t, \boldsymbol{\theta}), \quad \mathbf{x}(0, \boldsymbol{\theta}) = \mathbf{x_0}(\boldsymbol{\theta}), \\ \mathbf{y}(t, \boldsymbol{\theta}) = \mathbf{C}(\boldsymbol{\theta})\mathbf{x}(t, \boldsymbol{\theta}), \\ \Theta \subset \mathbb{R}^p,\ p \in \mathbb{N}, \quad \mathbf{A} : \Theta \to \mathbb{R}^{n \times n}, \quad \mathbf{C} : \Theta \to \mathbb{R}^{k \times n} \quad \text{and} \quad \mathbf{x_0} : \Theta \to \mathbb{R}^n.\end{gathered} \tag{4.2.1}$$

Using Definition 3.17, we define System (4.2.1) to have state evolution map $\mathbf{f}(\cdot, \boldsymbol{\theta}) = \mathbf{A}(\boldsymbol{\theta})\mathbf{x}(\cdot, \boldsymbol{\theta})$ and output map $\mathbf{h}(\cdot, \boldsymbol{\theta}) = \mathbf{C}(\boldsymbol{\theta})\mathbf{x}(\cdot, \boldsymbol{\theta})$.

Consider the requirements on $\mathbf{f}(\cdot, \boldsymbol{\theta})$ and $\mathbf{h}(\cdot, \boldsymbol{\theta})$ imposed by Condition 1 of Definition 3.17. The behaviour of $\mathbf{f}$ depends on that of the state vector $\mathbf{x}$. System (4.2.1) ensures that typically $\mathbf{x}(\cdot)$ is a sum of exponentials with constant coefficients, excepting the case where $\mathbf{A}$ has repeated eigenvalues. If $\mathbf{A}$ has all eigenvalues non-positive then $\mathbf{x}$ is bounded.[3] For real matrix $\mathbf{A}$ and real initial condition vector $\mathbf{x_0}(\boldsymbol{\theta})$, $\mathbf{x}(t, \boldsymbol{\theta})$ is real for all $t \in [0, t_1)$ and hence so is $\mathbf{f}(\cdot, \boldsymbol{\theta})$. Similarly, as $\mathbf{C}$ is a real matrix, $\mathbf{h}(\cdot, \boldsymbol{\theta}) = \mathbf{C}(\boldsymbol{\theta})\mathbf{x}(\cdot, \boldsymbol{\theta})$ is real-valued for all $t \in [0, t_1)$. The boundedness of $\mathbf{x}(\cdot)$ ensures the boundedness of $\mathbf{f}(\cdot, \boldsymbol{\theta})$ and $\mathbf{h}(\cdot, \boldsymbol{\theta})$. The nature of $\mathbf{x}(\cdot)$ means that it is infinitely differentiable with respect to t, and hence so are $\mathbf{f}(\cdot, \boldsymbol{\theta})$ and $\mathbf{h}(\cdot, \boldsymbol{\theta})$. Thus, the mappings $\mathbf{f}(\cdot, \boldsymbol{\theta})$ and $\mathbf{h}(\cdot, \boldsymbol{\theta})$ associated with the LTI system (4.2.1) satisfy Condition 1 of Definition 3.17. These observations are relevant whether one is considering a stand-alone LTI system, or the behaviour of a ULSS-1 (as we described earlier this section) on the time interval $t \in [0, t_1)$.

The same arguments and result apply to the LTI subsystem of the ULSS-1 that is in effect on the time interval $t \in [t_1, t_f)$. As a result, it is not necessary to include Condition 1 of Definition 3.17 in a discussion of global *a priori* identifiability of a ULSS-1 structure.

Let us consider how Condition 2 of Definition 3.17 — that application of the state evolution mapping to the initial state gives a non-zero result — should apply to a ULSS-1 structure. In the interests of generality, let us consider ULSS-1 for which parameters may be introduced before and after the switching event, as we defined in Section 3.7.

The natural extension of Condition 2 for this setting requires us to consider a ULSS-1 on the time intervals $[0, t_1)$ and $[t_1, t_f)$, and follows the principle outlined in Remark 3.4. The condition has two parts. The first requires that for almost all $\boldsymbol{\theta_1} \in \Theta_1$ the state of the ULSS-1 is not fixed at the initial condition $\mathbf{x_0}(\boldsymbol{\theta_1})$ for all $t \in (0, t_1)$. That is,

$$\dot{\mathbf{x}}(0, \boldsymbol{\theta}_1) = \mathbf{A}_1(\boldsymbol{\theta}_1)\mathbf{x_0}(\boldsymbol{\theta}_1) \neq \mathbf{0}, \quad \text{for almost all } \boldsymbol{\theta}_1 \in \Theta_1. \tag{4.2.2}$$

The second part follows similarly by considering the ULSS-1 on the interval $t \in (t_1, t_f)$ and requiring that for almost all $\boldsymbol{\theta} \in \Theta$ (where $\boldsymbol{\theta} = \langle \boldsymbol{\theta_1}, \boldsymbol{\theta_2} \rangle$, $\boldsymbol{\theta_1} \in \Theta_1$, $\boldsymbol{\theta_2} \in \Theta_2$) the state

[3] This condition is typical for linear compartmental systems, see Remark 3.11.

$\mathbf{x}(t, \boldsymbol{\theta})$ is not fixed at $\mathbf{x}(t_1, \boldsymbol{\theta})$ for all $t \in (t_1, t_f)$, that is,

$$\dot{\mathbf{x}}(t_1, \boldsymbol{\theta}) = \mathbf{A}_2(\boldsymbol{\theta}_2)\mathbf{x}(t_1, \boldsymbol{\theta}_1) \neq \mathbf{0}, \quad \text{for almost all } \boldsymbol{\theta} \in \Theta. \tag{4.2.3}$$

Now that we have adapted the conditions of Definition 3.17 to produce analogs that are suitable for ULSS-1 structures, we are able to propose a test of such a structure for global *a priori* identifiability.

Definition 4.1. Consider an ULSS-1 structure M where the switching event occurs at t_1, as described by Definition 3.32. Suppose the structure has time set $T = [0, t_f)$, $t_f \in \mathbb{R}_+$ and representative system $M(\boldsymbol{\theta})$ of the form shown in Equations (3.7.41) and (3.7.43).

Assume that $M(\boldsymbol{\theta})$ satisfies the conditions

$$\begin{aligned} \dot{\mathbf{x}}(0, \boldsymbol{\theta}_1) &= \mathbf{A}_1(\boldsymbol{\theta}_1)\mathbf{x_0}(\boldsymbol{\theta}_1) \neq \mathbf{0}, \quad && \text{for almost all } \boldsymbol{\theta}_1 \in \Theta_1, \\ \dot{\mathbf{x}}(t_1, \boldsymbol{\theta}) &= \mathbf{A}_2(\boldsymbol{\theta}_2)\mathbf{x}(t_1, \boldsymbol{\theta}_1) \neq \mathbf{0}, \quad && \text{for almost all } \boldsymbol{\theta} \in \Theta. \end{aligned} \tag{4.2.4}$$

Suppose we express the output of $M(\boldsymbol{\theta})$ as

$$\mathbf{y}(t, \boldsymbol{\theta}) = \begin{cases} \mathbf{g_1}\Big(\boldsymbol{\phi}_1(\boldsymbol{\theta}_1), t\Big), & t \in [0, t_1), \\ \mathbf{g_2}\Big(\boldsymbol{\phi}_2(\boldsymbol{\theta}), t\Big), & t \in [t_1, t_f), \end{cases} \tag{4.2.5}$$

where $\boldsymbol{\phi}_1$ and $\boldsymbol{\phi}_2$ are observational parameters of the output on the relevant time interval. We assume that these are uniquely determinable. Setting

$$\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq \Big(\boldsymbol{\phi_1}(\boldsymbol{\theta_1})^\top, \boldsymbol{\phi_2}(\boldsymbol{\theta})^\top\Big)^\top \tag{4.2.6}$$

provides a vector of invariants we require for testing M for global a priori identifiability following Definition 3.19.

The test of a ULSS-1 structure for global *a priori* identifiability described in Definition 4.1 hinges on the determination of $\boldsymbol{\phi}$, the set of invariants present in the output $\mathbf{y}$. The nature of linear switching systems means that obtaining $\boldsymbol{\phi}_2$ is not trivial in general. We discuss this problem in the next section.

4.3 The mechanics of testing a ULSS-1 structure for global *a priori* identifiability

In Section 1.4 we noted the relatively minor coverage of testing continuous-time LSS structures for global *a priori* identifiability. We suspect that this explains the literature's lack of interest in obtaining the invariants present in the output of a ULSS-1 structure. We propose an approach in which we treat a ULSS-1 structure as defining two LTI structures. There is a well-developed theory for obtaining the invariants of these, as described in Section 3.5.1.1. We will adapt these methods for our context. We achieve this by extracting the two LTI subsystems of a ULSS-1 representative system from their context and using them to define LTI structures.

4.3.1 Translating a ULSS-1 structure M into LTI structures $M^{[1]}$ and $M^{[2]}$

Consider system structure M with parameter set Θ comprised of uncontrolled LSSs of one switching event as described by (3.7.41) and (3.7.43). For some unspecified $\boldsymbol{\theta} \in \Theta$, let $M(\boldsymbol{\theta})$ be the representative system of M. As ultimately we will consider the idealised output of M (as described on Page 12), we require that systems in M are defined for time set $T = \bar{\mathbb{R}}_+$.

Let us define an uncontrolled LTI structure $M^{[1]}$ with parameter set $\Theta_1 \subseteq \Theta$ defined for $T = [0, t_1)$. Let $\boldsymbol{\theta_1} \in \Theta_1$ be some arbitrary parameter vector. We use the state $\mathbf{x}$ and output $\mathbf{y}$ of $M(\boldsymbol{\theta})$ for $t \in [0, t_1)$ to define the state $\mathbf{x}^{[1]}$ and output $\mathbf{y}^{[1]}$ of $M^{[1]}(\boldsymbol{\theta}_1)$, respectively.[4] That is, the behaviour of $M(\boldsymbol{\theta})$ for $t \in [0, t_1)$ is encapsulated by $M^{[1]}(\boldsymbol{\theta}_1)$.

Let us similarly capture the behaviour of $M(\boldsymbol{\theta})$ for $t \in [t_1, \infty)$ in an uncontrolled LTI system. One may use $M(\boldsymbol{\theta})$ for $t \in [t_1, \infty)$ to define an uncontrolled LTI system $M^{[2]}(\boldsymbol{\theta})$ having $T = \bar{\mathbb{R}}_+$ which has state $\mathbf{x}^{[2]}$ and output $\mathbf{y}^{[2]}$ defined by $\mathbf{x}$ and $\mathbf{y}$ of $M(\boldsymbol{\theta})$

[4] By distinguishing between $\boldsymbol{\theta}$ and $\boldsymbol{\theta}_1$ we allow for the case where some parameters in $M(\boldsymbol{\theta})$ do not enter into the response before the switching time. These should not appear in the parameter vector for $M^{[1]}$ as they certainly cannot be estimated from $\mathbf{y}^{[1]}$.

respectively for $t \in [t_1, \infty)$. We define the initial state of $M^{[2]}(\boldsymbol{\theta})$ as

$$\mathbf{x}^{[\mathbf{2}]}(0, \boldsymbol{\theta}_1) = \mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1) \tag{4.3.7}$$

to preserve the dependence of $\mathbf{x}(t_1, \boldsymbol{\theta})$ (equivalently $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1)$) on the dynamics of $M(\boldsymbol{\theta})$ for $t \in (0, t_1)$. This allows us to relate $M^{[2]}$ to $M^{[1]}$, rather than to M directly.

By defining time sets $T_1 = [0, t_1)$ and $T_2 = \bar{\mathbb{R}}_+$, we may express the state and output of each of $M^{[1]}(\boldsymbol{\theta_1})$ and $M^{[2]}(\boldsymbol{\theta})$ as $\mathbf{x}^{[\mathbf{i}]} : T_i \to X$ and $\mathbf{y}^{[\mathbf{i}]} : T_i \to Y$ for $i = 1, 2$. We show these explicitly as

$$\mathbf{x}^{[\mathbf{1}]}(t, \boldsymbol{\theta}_1) = e^{\mathbf{A_1}(\boldsymbol{\theta_1})t}\mathbf{x_0}(\boldsymbol{\theta_1}), \qquad \mathbf{y}^{[\mathbf{1}]}(t, \boldsymbol{\theta}_1) = \mathbf{C_1}(\boldsymbol{\theta_1})\mathbf{x}^{[\mathbf{1}]}(t, \boldsymbol{\theta}_1), \tag{4.3.8}$$

and using (4.3.7),

$$\mathbf{x}^{[\mathbf{2}]}(t, \boldsymbol{\theta}) = e^{\mathbf{A_2}(\boldsymbol{\theta_2})t}\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1), \quad \mathbf{y}^{[\mathbf{2}]}(t, \boldsymbol{\theta}) = \mathbf{C_2}(\boldsymbol{\theta_2})\mathbf{x}^{[\mathbf{2}]}(t, \boldsymbol{\theta}). \tag{4.3.9}$$

We note that defining

$$\tilde{\mathbf{x}}_\mathbf{2}(t, \boldsymbol{\theta}) = \begin{cases} \mathbf{x}^{[\mathbf{2}]}(t - t_1, \boldsymbol{\theta}), & \forall t \in [t_1, \infty), \\ \mathbf{0}, & \forall t \in [0, t_1), \end{cases}$$

and

$$\tilde{\mathbf{y}}_\mathbf{2}(t, \boldsymbol{\theta}) = \mathbf{C_2}(\boldsymbol{\theta_2})\tilde{\mathbf{x}}_\mathbf{2}(t, \boldsymbol{\theta}), \quad \forall t \in [0, \infty),$$

allows us to express the output of $M(\boldsymbol{\theta})$ as

$$\mathbf{y}(\cdot, \boldsymbol{\theta}) = \mathbf{y}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta_1})\left(\mathcal{H}_0 - \mathcal{H}_{t_1}\right) + \tilde{\mathbf{y}}_\mathbf{2}(\cdot, \boldsymbol{\theta}). \tag{4.3.10}$$

This demonstrates that $M^{[1]}(\boldsymbol{\theta}_1)$ and $M^{[2]}(\boldsymbol{\theta})$ provide the same output — and hence the same conditions on the parameters — as $M(\boldsymbol{\theta})$.

Remark 4.1. If an ULSS-1 structure M is piecewise positive (piecewise compartmental) then each of the LTI subsystems of systems in M are positive (compartmental), and hence, so are $M^{[1]}$ and $M^{[2]}$.

The ability of $M^{[1]}$ and $M^{[2]}$ to reproduce M's response suggests an approach to our problem of how to obtain a vector of response invariants (say $\boldsymbol{\phi}$) associated with M.

Proposition 4.1. *The problem of obtaining* $\boldsymbol{\phi}$ *(as defined by* (4.2.6)*) from the output of ULSS-1* $M(\boldsymbol{\theta})$ *is equivalent to the problem of determining the response invariants of the LTI systems* $M^{[1]}(\boldsymbol{\theta}_1)$ *and* $M^{[2]}(\boldsymbol{\theta})$*.*

Following Proposition 4.1, if we wish to test M for global *a priori* identifiability via $M(\boldsymbol{\theta})$, then we are required to determine the invariants $\boldsymbol{\phi}_1(\boldsymbol{\theta}_1)$ and $\boldsymbol{\phi}_2(\boldsymbol{\theta})$ present in responses $\mathbf{y}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta}_1)$ of $M^{[1]}(\boldsymbol{\theta}_\mathbf{1})$ and $\mathbf{y}^{[\mathbf{2}]}(\cdot, \boldsymbol{\theta})$ of $M^{[2]}(\boldsymbol{\theta})$, respectively. We consider this problem in the next subsection.

4.3.2 Obtaining response invariants from $M^{[1]}$ and $M^{[2]}$

As we noted in Section 3.6.4, response invariants are features of systems from a generically minimal structure. Hence, it is appropriate to establish that a structure has this property (or derive from it a structure that does) before proceeding to obtain invariants. Given Proposition 4.1, we must apply this logic to $M^{[1]}$ and $M^{[2]}$.

Recall the testing of a LTI structure $\mathcal{M}$ for generic minimality described in Section 3.6.3.4. The method we outlined there proceeds by inspection of the Laplace transform of response of $\mathcal{M}(\boldsymbol{\theta})$. For a compartmental ULSS-1 structure M with representative system $M(\boldsymbol{\theta})$, a variation on this method seems appropriate. Suppose one applies a logic similar to that of Section 3.6.3.4 in testing the compartmental LTI structures $M^{[1]}$ and $M^{[2]}$ for generic minimality. The test requires that each structure is defined for $T = \bar{\mathbb{R}}_+$. This is no greater an idealisation than is often used when defining generic properties such as global *a priori* identifiability.[5]

Following this convention, let us require that $M^{[1]}$ — like $M^{[2]}$ — is defined for $T = \bar{\mathbb{R}}_+$, and test $M^{[1]}$ and $M^{[2]}$ for generic minimality according to Section 3.6.3.4. This requires an expression for each of $\mathcal{L}\{\mathbf{y}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta}_\mathbf{1})\}(s)$ and $\mathcal{L}\{\mathbf{y}^{[\mathbf{2}]}(\cdot, \boldsymbol{\theta})\}(s)$.

[5] Another justification for the assumption that an infinite output is obtainable is given by the theory of analytic continuations; as $y^{[1]}$ is analytic and known for all $t \in [0, t_1)$ $(t_1 > 0)$, it is known for all $t \in \bar{\mathbb{R}}_+$.

Definition 4.2 (Laplace transform of $\mathbf{y^{[1]}}$ and $\mathbf{y^{[2]}}$)**.** Suppose M is an ULSS-1 structure from which uncontrolled LTI structures $M^{[i]}$ $(i = 1, 2)$ are obtained. Let $M^{[i]}$ have representative system $M^{[i]}(\boldsymbol{\theta}^{(i)})$. System $M^{[1]}(\boldsymbol{\theta}_1)$ has state $\mathbf{x^{[1]}}$ and output $\mathbf{y^{[1]}}$ as shown by (4.3.8), system $M^{[2]}(\boldsymbol{\theta}^{(2)})$ has state $\mathbf{x^{[2]}}$ and output $\mathbf{y^{[2]}}$ as in (4.3.9).

On defining

$$\mathbf{K}_i(\boldsymbol{\theta}_i) \triangleq \mathbf{C}_i(\boldsymbol{\theta}_i)\big(s\mathbf{I}_n - \mathbf{A}_i(\boldsymbol{\theta}_i)\big)^{-1}, \quad i = 1, 2, \tag{4.3.11}$$

the Laplace transform of $\mathbf{y^{[i]}}$ $(i = 1, 2)$ in its unprocessed form (recall Section 3.6.3.4) is

$$\begin{aligned} \mathcal{L}\{\mathbf{y^{[1]}}(\cdot, \boldsymbol{\theta_1})\}(s) &= \mathbf{K}_1(\boldsymbol{\theta_1})\mathbf{x_0}(\boldsymbol{\theta_1})\,, \qquad \forall s \in \mathrm{H}_{k_1}\,, \\ \mathcal{L}\{\mathbf{y^{[2]}}(\cdot, \boldsymbol{\theta})\}(s) &= \mathbf{K}_2(\boldsymbol{\theta_2})\mathbf{x^{[1]}}(t_1, \boldsymbol{\theta_1})\,, \quad \forall s \in \mathrm{H}_{k_2}\,, \end{aligned} \tag{4.3.12}$$

where H_{k_i} represents the domain of convergence of $\mathcal{L}\{\mathbf{y^{[i]}}\}$. When M is also piecewise compartmental, elements of $\mathcal{L}\{\mathbf{y^{[1]}}(\cdot, \boldsymbol{\theta_1})\}$ as in (4.3.12) have the same form as the Laplace transform of the scalar output of a general compartmental uncontrolled LTI system shown in (3.6.32). We will see that elements of $\mathcal{L}\{\mathbf{y^{[2]}}\}$ deviate from that general form.

Given that we are interested in typical flow-cell optical biosensor experiments in this thesis, some restrictions are appropriate. Let us start by considering M having scalar output. Accordingly, setting $i = 1$ in an expression of the form of (4.3.12) gives

$$\begin{gathered} \mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta_1})\}(s) = \frac{\varphi^{[1]}_{r_1+p_1}(\boldsymbol{\theta_1})s^{p_1} + \cdots + \varphi^{[1]}_{r_1}(\boldsymbol{\theta_1})}{s^{r_1} + \varphi^{[1]}_{r_1-1}(\boldsymbol{\theta}_1)s^{r_1-1} + \cdots + \varphi^{[1]}_0(\boldsymbol{\theta}_1)}, \quad \forall s \in \mathrm{H}_{k_1}, \\ \varphi^{[1]}_j(\boldsymbol{\theta_1}) \triangleq \varphi^{[1]}_j(\mathbf{x_0}(\boldsymbol{\theta_1}), \boldsymbol{\theta_1}) \quad j = r_1, \ldots, r_1 + p_1, \\ r_1 \in \{1, \ldots, n\}, \quad p_1 \in \{0, \ldots, r_1 - 1\}. \end{gathered} \tag{4.3.13}$$

In considering $\mathcal{L}\{y^{[2]}\}$ as defined by (4.3.12) for $i = 2$, the expression depends on the parameter vector $\langle \boldsymbol{\theta_1}, \boldsymbol{\theta_2} \rangle$. In a structure representing an experiment of one association phase and one dissociation phase, all parameters are present in the first subsystem of the

structure's representative system. Under this restriction, $\langle \boldsymbol{\theta_1}, \boldsymbol{\theta_2} \rangle \equiv \boldsymbol{\theta}$. Hence,

$$\begin{gathered}\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s) = \frac{\varphi^{[2]}_{r_2+p_2}(\boldsymbol{\theta}) s^{p_2} + \cdots + \varphi^{[2]}_{r_2}(\boldsymbol{\theta})}{s^{r_2} + \varphi^{[2]}_{r_2-1}(\boldsymbol{\theta_2}) s^{r_2-1} + \cdots + \varphi^{[2]}_{0}(\boldsymbol{\theta_2})}, \quad \forall s \in \mathrm{H}_{k_2}, \\ \varphi^{[2]}_j(\boldsymbol{\theta}) \triangleq \varphi^{[2]}_j(\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta_1}), \boldsymbol{\theta_2}), \quad j = r_2, \ldots, r_2 + p_2, \\ r_2 \in \{1, \ldots, n\}, \quad p_2 \in \{0, \ldots, r_2 - 1\}.\end{gathered} \tag{4.3.14}$$

In Equations (4.3.13) and (4.3.14) we mimic the notation used for a general compartmental uncontrolled LTI system in Equation (3.6.32) with one important difference. Note that expressions $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta_1})\}$ and $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}$ are in an unprocessed form which is not necessarily the canonical form. For this reason, we use φ (rather than ϕ) in (4.3.13) and (4.3.14) to show that the coefficients of s are not necessarily invariants.

Remark 4.2. The expression given in (4.3.12) provides a description of the dependence of $\mathcal{L}\{\mathbf{y}^{[\mathbf{i}]}\}$ on parameters for arbitrary $i \in \mathbb{N}$. As such, it is also applicable to ULSS structures of more than one switching event.

We have that $M^{[1]}(\boldsymbol{\theta_1})$ is an uncontrolled compartmental LTI system with initial conditions that — whilst unknown — are not functions of time. For such a structure, we have prescribed steps for obtaining the canonical form of $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta}_1)\}$, as given in Definition 3.26. Towards this, we can employ an approach to detecting factors common to the numerator and denominator of $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta}_1)\}$ as described in Remark 3.10. Having put $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta}_1)\}(s)$ into the canonical form, the collection of coefficients of s gives $\boldsymbol{\phi_1}(\boldsymbol{\theta_1})$.

In considering $M^{[2]}(\boldsymbol{\theta})$, we cannot proceed to obtain the canonical form of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}$ in the same manner as for $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta_1})\}$ in general.[6] This is a result of the numerator terms of $\mathcal{L}\{y^{[2]}\}(s)$ depending on $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1)$, which causes numerator factors to have a different form to the denominator factors. Thus, the approach of Remark 3.10 is unsuitable for obtaining a numerator and denominator of $\mathcal{L}\{y^{[2]}\}(s)$ that are relatively prime. We note

[6] From this point onward a notational abuse may be employed to represent a term such as $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$ more simply as $\mathcal{L}\{y^{[2]}\}$ in the interests of brevity.

that a general method of testing ULSS-1 structures would seem to require a means of putting $\mathcal{L}\{y^{[2]}\}(s)$ into the canonical form.[7]

We illuminate the possible effect of $\mathbf{x}^{[1]}$ on the canonical form of $\mathcal{L}\{y^{[2]}\}$ through a schematic for the evolution of the state of a ULSS-1 given in Figure 4.3.3. Suppose the subsystems of any ULSS-1 in the structure notionally have states in $\mathbb{R}^3_+$. Let the final state of the first phase of the ULSS-1 be $\boldsymbol{\xi}$, which occurs at time t_1. This becomes the initial state of the second phase. Consider the case where, as a result of this initial state, states reached by the second phase LTI system are restricted to some space of lower dimension than $\mathbb{R}^3_+$. In Figure 4.3.3 we show this by restricting the state to a plane following the switching event. In such a case, the dynamics of the second phase of the ULSS-1 are not comparable to that of the first phase. A particular example of this effect is when $\boldsymbol{\xi}$ is the equilibrium state of the second subsystem of the ULSS-1, in which case the state — and hence response — is fixed for all time after the switching event. If such a property holds for almost all $\boldsymbol{\theta} \in \Theta$ for a given time, the Laplace transform of $y^{[2]}(\cdot, \boldsymbol{\theta})$ will not be a rational function with cubic denominator in general, as we may expect from the dimensions of the system matrices. However, t_1 itself is not necessarily fixed, and hence ascertaining the canonical form of $\mathcal{L}\{y^{[2]}\}$ in the *a priori* identifiability analysis of a ULSS-1 structure presents a different challenge to that presented by $\mathcal{L}\{y^{[1]}\}$.

If we cannot determine the canonical form of $\mathcal{L}\{y^{[2]}\}(s)$, we are unable to obtain $\boldsymbol{\phi}_2$ for use in testing some ULSS-1 structure M for global *a priori* identifiability as per Definition 4.1. This limitation on the parameter information obtainable from the output of representative system $M(\boldsymbol{\theta})$ could conceivably limit the predictive power of the test. To clarify this, suppose only conditions on $\boldsymbol{\theta}$ imposed by $\boldsymbol{\phi_1}$ are available. Further suppose the set $\mathcal{I}(M, \boldsymbol{\phi_1})$ (defined by analogy to $\mathcal{I}(M, \boldsymbol{\phi})$ in (3.5.20) of Definition 3.19) contains more than one element. Then, the test gives merely an upper bound on the number of solutions of $\mathcal{I}(M, \boldsymbol{\phi})$, rather than the unambiguous classification we expect from $\mathcal{I}(M, \boldsymbol{\phi})$.

[7] The earlier considerations of a structure for the simple bimolecular interaction produced an unprocessed $\mathcal{L}\{y^{[2]}\}(s)$ which was already of the canonical form. As such, it was not necessary to posit a method for determining the canonical form of $\mathcal{L}\{y^{[2]}\}(s)$ at that time.

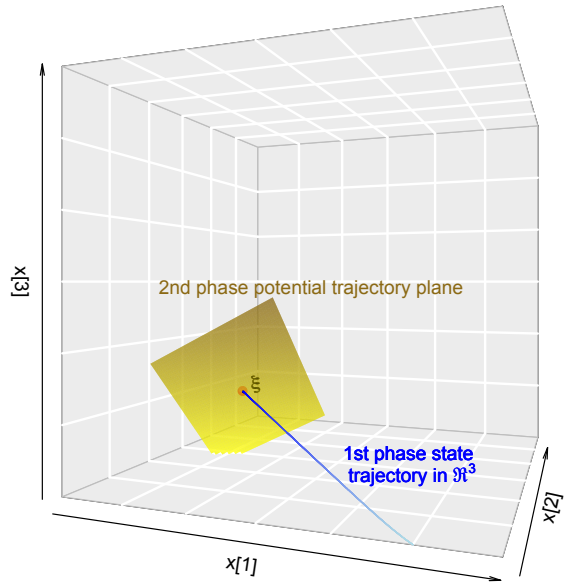


Figure 4.3.3: A schematic of feasible states for a ULSS-1 with the state space for the second phase dependent on the final state reached by the first phase. While the state of the first subsystem evolves in $\mathbb{R}^3_+$, as a consequence of the final state of the first phase $\boldsymbol{\xi}$, the states reached by the second subsystem of the ULSS-1 are restricted to a plane.

At this point, it appears that we must devise a method or obtaining $\boldsymbol{\phi}_2$ if we wish to test a ULSS-1 structure for global *a priori* identifiability. We may consider this a 'classical approach' to the problem. However, this is not the only way to approach the matter. Let us consider an alternative method which is sufficient for classifying a ULSS-1 structure as globally *a priori* identifiable. Its ease of use makes it a reasonable first step in structure classification. Further, the process will subsequently provide a check on results obtained by the more sophisticated — and correspondingly more laborious — methods in support of a classical approach which follow.

4.4 A method sufficient for inferring global *a priori* identifiability of a ULSS-1 structure

This section is based on material first presented in Whyte [92], subsequently refined in Whyte [94].

Let us consider a test of a ULSS-1 structure M for global *a priori* identifiability that does not require the canonical form of $\mathcal{L}\{y^{[2]}\}$. Instead, the test considers the unprocessed form of $\mathcal{L}\{y^{[2]}\}$ and all rational functions that are obtainable from it if pole-zero cancellation is assumed to occur. One of these alternative forms is the canonical form of $\mathcal{L}\{y^{[2]}\}$.

Suppose one of the alternative forms of $\mathcal{L}\{y^{[2]}\}$ is treated as the canonical form of $\mathcal{L}\{y^{[2]}\}$. We use the collection of non-numerical coefficients of this rational function to form a vector of moment invariants (although these may not be the actual moment invariants of the structure). We combine these with $\boldsymbol{\phi}_1$ obtained from $\mathcal{L}\{y^{[1]}\}$ to form a vector of invariants $\hat{\boldsymbol{\phi}}$. Using $\hat{\boldsymbol{\phi}}$ we can determine a set $\mathcal{I}(M, \hat{\boldsymbol{\phi}})$ that is similar to $\mathcal{I}(M, \boldsymbol{\phi})$ in (3.5.20). From $\mathcal{I}(M, \hat{\boldsymbol{\phi}})$ we obtain the result of a hypothetical test of the structure M for global *a priori* identifiability.

Let us apply the process described above to all of the alternative forms of $\mathcal{L}\{y^{[2]}\}$. Suppose that all of these alternatives lead to the same conclusion when used in the classification of M. Further suppose that this conclusion is that M is globally or locally *a priori* identifiable. Then, this is the classification that would be obtained from using the canonical (but unknown) form of $\mathcal{L}\{y^{[2]}\}$. Hence, the process outlined is sufficient to classify M. Less informative results are also possible. This outline is developed below in the Structure Classification Under Incomplete Information (SCUII) algorithm.

SCUII Algorithm. Consider a ULSS-1 structure M having representative system $M(\boldsymbol{\theta})$. Let this determine the construction of systems $M^{[1]}(\boldsymbol{\theta_1})$ and $M^{[2]}(\boldsymbol{\theta})$ that are representative of uncontrolled LTI structures, as described in Section 4.3.1.

1. For $M^{[1]}(\boldsymbol{\theta_1})$, determine the canonical form of $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta_1})\}(s)$. Collect the coefficients to form the vector of invariants $\boldsymbol{\phi}_1(\boldsymbol{\theta_1})$. If all parameters of $M(\boldsymbol{\theta})$ are introduced in $M^{[1]}(\boldsymbol{\theta_1})$, proceed to step 2. Otherwise, proceed to step 3.

2. Calculate $\mathcal{I}(M^{[1]}, \boldsymbol{\phi_1})$ and classify $M^{[1]}$, (and hence M using incomplete information) according to Definition 3.17. If M is classified as globally *a priori* identifiable (or as locally *a priori* identifiable, and this classification is adequate given the intended use of the structure or an anticipated means of restricting the plausible range parameters), stop. Otherwise, proceed to step 3.

3. For $M^{[2]}(\boldsymbol{\theta})$, derive the unprocessed form of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$ and each of the alternative forms obtainable from it by pole-zero cancellation. Enumerate these candidates for the canonical form of $\mathcal{L}\{y^{[2]}\}$ from 1 to N. (Note that if there are no alternatives to the unprocessed form of $\mathcal{L}\{y^{[2]}\}$ then it is unambiguously the canonical form of $\mathcal{L}\{y^{[2]}\}$.)

4. For $i = 1, \ldots, N$, obtain coefficients of s in the denominator of form i of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$, combine these with $\boldsymbol{\phi}_1(\boldsymbol{\theta_1})$ to form $\hat{\boldsymbol{\phi}}(\boldsymbol{\theta})$, and for $\boldsymbol{\theta} \in \Theta$, calculate

$$\hat{\mathcal{I}}(M, \hat{\boldsymbol{\phi}})_i \triangleq \left\{ \boldsymbol{\theta}' \in \Theta : \hat{\boldsymbol{\phi}}(\boldsymbol{\theta}') = \hat{\boldsymbol{\phi}}(\boldsymbol{\theta}) \right\}. \tag{4.4.15}$$

5. Select the $\hat{\mathcal{I}}(M, \hat{\boldsymbol{\phi}})_i$ for $i = 1, \ldots, N$ that have the greatest number of elements and define one of these as $\mathcal{I}_{\max}$. (As it is the number of elements in the set rather than the membership of the set which is important, the choice of $\mathcal{I}_{\max}$ from the candidates can be made arbitrarily.)

6. If for almost all $\boldsymbol{\theta} \in \Theta$:

   - $|\mathcal{I}_{\max}| = 1$: M is globally *a priori* identifiable,
   - $\mathcal{I}_{\max}$ is denumerable: M is at worst locally *a priori* identifiable,
   - $\mathcal{I}_{\max}$ is uncountably infinite: no judgement on M is possible with this algorithm.

Remark 4.3. In effect, the SCUII Algorithm gives the most unfavourable classification of M possible given the range of possibilities for the canonical form of $\mathcal{L}\{y^{[2]}\}$.

A graphical impression of the SCUII Algorithm is given in Figure 4.4.4. The SCUII Algorithm is applied to a test case in Chapter 5.

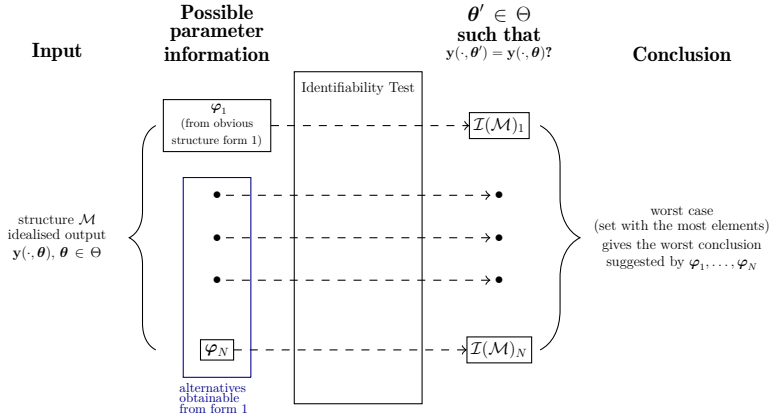


Figure 4.4.4: A diagrammatic representation of the SCUII Algorithm.

The SCUII Algorithm was applied to a test case in Whyte [94]. This application showed that the algorithm was able to circumvent the difficulties encountered by the previous approaches to classifying a flow-cell optical biosensor interaction model structure. However, classification of a structure using the SCUII Algorithm may give a pessimistic result compared to what would be obtained by using the canonical form of $\mathcal{L}\{y^{[2]}\}(s)$ in a test of a system structure for global *a priori* identifiability. A general method for classifying a ULSS-1 structure precisely — rather than conservatively — requires progress with the problem of how to obtain invariants from $\mathcal{L}\{y^{[2]}\}(s)$.

The following section addresses this problem by considering a method for determining the canonical form of $\mathcal{L}\{y^{[2]}\}(s)$, and other methods aiming to deduce whether or not $M^{[2]}$ is generically minimal. Having developed these strategies, we are able to propose a scheme for testing a continuous-time ULSS-1 structure for global *a priori* identifiability in Section 4.6 which is an advance on that previously available.

4.5 Approaches to determining whether $M^{[2]}$ is generically minimal

The difficulty of directly testing $M^{[2]}$ for generic minimality suggests an alternative approach of aiming to infer the property by consideration of a less demanding problem. In this section we propose an approach to the problem of testing $M^{[2]}$ for generic minimality by addressing a sightly different question.

Question 4.1. Given model structure M of systems of n state variables, having output y and parameter set Θ, for which parameter values $\boldsymbol{\theta} \in \Theta$ is $M(\boldsymbol{\theta})$ indistinguishable (see Section 3.5.4.1) from a system of fewer than n state variables?

If the only solutions to Question 4.1 belong to a subset of Θ of measure zero, then $M(\boldsymbol{\theta})$ is not indistinguishable from a structure of systems of fewer states almost everywhere. Hence, M is generically minimal.

Possibly the most obvious means of addressing Question 4.1 is to consider the conditions under which pole-zero cancellation occurs in $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$. This is inspired by the use of such an approach in LTI structures.

4.5.1 Consideration of pole-zero cancellation in $\mathcal{L}\{y^{[2]}\}(s)$

Definition 3.30 inspires an alternative form of Question 4.1 which is useful when M is a LTI structure.

Question 4.2. For M an LTI structure, Question 4.1 is equivalent to asking:

For which parameter values $\boldsymbol{\theta} \in \Theta$ does pole-zero cancellation occur in the unprocessed form of $\mathcal{L}\{y\}(s, \boldsymbol{\theta})$?

We present an approach to finding solutions to Question 4.2 in Proposition 4.2.

Proposition 4.2. *Consider the unprocessed* $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s)$ *given by* (4.3.14) *with its denominator in factored form,*

$$\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s) = \frac{\mathbf{C_2}(\boldsymbol{\theta}_2)\,\mathrm{adj}(s\mathbf{I}-\mathbf{A_2}(\boldsymbol{\theta}_2))\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)}{\prod_{k=1}^{p_2}(s-s_k(\boldsymbol{\theta}_2))}, \tag{4.5.16}$$

where $s_k(\boldsymbol{\theta}_2)$, $k=1,\ldots,p_2$ *are zeros of the denominator of* (4.5.16). *Pole-zero cancellation occurs in* $\mathcal{L}\{y^{[2]}\}$ *if for some* $\boldsymbol{\theta}\in\Theta$ *any* s_k $(k=1,\ldots,p_2)$ *is a zero of the numerator of* (4.5.16). *Hence Question 4.2 is answered by finding all* $\boldsymbol{\theta}\in\Theta$ *which satisfy at least one of the* **cancellation conditions**

$$\mathbf{C_2}(\boldsymbol{\theta}_2)\,\mathrm{adj}(s_k(\boldsymbol{\theta}_2)\mathbf{I}-\mathbf{A_2}(\boldsymbol{\theta}_2))\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)=0, \quad k=1,\ldots,p_2\,. \tag{4.5.17}$$

Remark 4.4. Forming the equations of System (4.5.17) requires an explicit expression for $\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)$, requiring algebraic solution of its associated differential equation system. Further, $\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)$ has different forms depending on the multiplicity of eigenvalues of $\mathbf{A_1}$, which is unknown in general. Taken together these points suggest that solving (4.5.17) may require the generation and solution of multiple sets of conditions, a process that is likely to become more laborious as the number of compartments (or state variables) in a structure increases.

The potential difficulty of the application of Proposition 4.2 to Question 4.2 motivates a less algebraically complex approach to the problem. The enquiry we pose here is: is it possible to show that pole-zero cancellation in the unprocessed form of $\mathcal{L}\{y^{[2]}\}$ only occurs for a subset of Θ of measure zero by leaving $\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)$ terms as implicit functions of $\boldsymbol{\theta}_1$ in the cancellation conditions of System (4.5.17)? If so, this new approach shows that $M^{[2]}$ is generically minimal.

Our decision to treat $\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)$ as a variable that is not an explicit function of parameters means that properties of $\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)$ are obscured, such as the relationships between components. To compensate for this, it is appropriate to impose certain constraints on $\mathbf{x}^{[\mathbf{1}]}(t_1,\boldsymbol{\theta}_1)$ that were not required in Proposition 4.2. We illustrate this matter through the framework we present in Proposition 4.3.

Proposition 4.3. *Given an LTI structure* $M^{[2]}$ *and the cancellation conditions* (4.5.17)*, replace* $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1) \in X \subseteq \mathbb{R}^n$ *in these equations by* $\boldsymbol{\xi} = (\xi_1, \ldots, \xi_n)^\top \in X$ *to give a system of conditions that does not require an explicit expression for the components of* $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1)$*. Propose a system of conditions which includes the new cancellation conditions and any constraints on* $\boldsymbol{\theta}$ *and* $\boldsymbol{\xi}$ *that arise from requiring feasible solutions. For example, these may be* $\boldsymbol{\theta} \in \Theta$*,* $\boldsymbol{\xi} \in X$*, and any other conditions that arise from requiring solutions that are physically sensible. For the purpose of illustration, we may cast the problem in the following general manner.*

For p_2 *the degree of the denominator of* $\mathcal{L}\{y^{[2]}\}$*, for each of* $k = 1, \ldots, p_2$*, determine the* $\boldsymbol{\theta}, \boldsymbol{\xi}$ *that satisfy*

$$\begin{array}{ll} \text{cancellation:} & \mathbf{C_2}(\boldsymbol{\theta}_2)\text{adj}(s_k(\boldsymbol{\theta}_2)\mathbf{I} - \mathbf{A_2}(\boldsymbol{\theta}_2))\boldsymbol{\xi} = 0, \\ \textit{and both} & \\ \text{feasibility:} & \boldsymbol{\theta} \in \Theta \quad \textit{and} \quad \boldsymbol{\xi} \in X, \\ \text{physicality:} & \text{as dictated by the physical system under consideration.} \end{array} \tag{4.5.18}$$

The simultaneous consideration of the solution sets of the p_2 *systems described by* (4.5.18) *leads to two instructive possibilities.*

*(*P1*) Suppose at least one of the systems associated with a cancellation condition in* (4.5.18) *is satisfied for almost all combinations of initial state and system parameters. Hence, pole-zero cancellation of the factor corresponding to any such cancellation condition occurs in* $\mathcal{L}\{y^{[2]}\}$ *for almost all combinations of initial state and system parameters. As a result, the unprocessed version of* $\mathcal{L}\{y^{[2]}\}$ *is not the canonical form. Hence,* $M^{[2]}$ *is not generically minimal. The test of* M *for global a priori identifiability according to Definition 3.19 cannot proceed.*

*(*P2*) If* (P1) *does not hold, the approach has shown that* $M^{[2]}$ *is generically minimal. As a result,* $\boldsymbol{\phi}_2$ *is obtainable directly from* $M^{[2]}$ *for use in the test of* M *for global a priori identifiability.*

Remark 4.5. Let us consider some specific examples of (4.5.18). For n state LTI structure

$M^{[2]}$ representing a physical system which does not receive inputs and which relates non-negative quantities, following the template of System (4.5.18) the problem is:

For each of $k = 1, \ldots, p_2$, determine $\boldsymbol{\theta}$, $\boldsymbol{\xi}$ that satisfy

$$\begin{aligned} &\text{cancellation:} \quad \mathbf{C_2}(\boldsymbol{\theta}_2)\text{adj}(s_k(\boldsymbol{\theta}_2)\mathbf{I} - \mathbf{A_2}(\boldsymbol{\theta}_2))\boldsymbol{\xi} = 0, \\ &\text{and both} \\ &\text{feasibility:} \quad \boldsymbol{\theta} \in \Theta \quad \text{and} \quad \xi_i \geq 0 \text{ for } i \in 1, \ldots, n, \\ &\text{physicality:} \quad \sum_{i \in \mathcal{S}} \xi_i \leq \sum_{i=1}^{n} \mathbf{x_0}_i(\boldsymbol{\theta_1}), \quad \text{for } \mathcal{S} \subseteq \{1, \ldots, n\}, \end{aligned} \tag{4.5.19}$$

where $\mathcal{S}$ denotes the set of states that explicitly appear in $\mathcal{L}\{y^{[2]}\}$.

Further conditions in (4.5.19) or refinements may arise from the nature of the model structure under consideration. For the case of a closed, uncontrolled compartmental system of n states, it may be appropriate to replace the physicality condition of (4.5.19) with $\sum_{i=1}^{n} \xi_i = \sum_{i=1}^{n} \mathbf{x_0}_i(\boldsymbol{\theta_1})$. This makes explicit the property that the total amount of mass in the system at any time point is equal to the total mass present in the system initially. When considering an LTI compartmental structure, one can require non-positivity of the eigenvalues of the $\mathbf{A}$ matrix of the structure, see Remark 3.11.

If the result of the process given in Proposition 4.3 is as described in (P2), this is a useful result for the analysis of $M^{[2]}$. However, if the result is neither (P1) nor (P2), the attempt to simplify the problem of determining whether cancellation of factors can occur in $\mathcal{L}\{y^{[2]}\}$ has failed.

In the following section we draw together the developments of this chapter to describe a process for testing a ULSS-1 structure for global *a priori* identifiability.

4.6 A more general scheme for testing a ULSS-1 structure for global *a priori* identifiability

Remark 4.6. When we test a structure for global *a priori* identifiability, we expect to obtain all of the invariants from a response function. However, this may not be a simple task. Recall that if we wish to test some ULSS-1 structure M, our method requires $M^{[2]}(\boldsymbol{\theta})$. The response of this LTI system is determined by invariants that depend on $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1)$ (that is, those in the numerator of $\mathcal{L}\{y^{[2]}\}$). In order to obtain these, we must solve the differential equation system for the state of $M^{[1]}$, and subsequently manage the issues noted in Remark 4.4. It may be that we cannot readily use invariants that appear in the numerator of $\mathcal{L}\{y^{[2]}\}$).

However, the denominator coefficients of $\mathcal{L}\{y^{[2]}\}$ do not require an explicit expression for $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1)$. In particular, if $M^{[2]}$ is generically minimal then the unprocessed form of $\mathcal{L}\{y^{[2]}\}$ is the canonical form. In this case, the denominator of $\mathcal{L}\{y^{[2]}\}$ readily supplies some of the components of $\boldsymbol{\phi}_2$.

We choose to investigate if it is possible to judge a ULSS-1 structure as globally *a priori* identifiable without requiring the invariants in $\boldsymbol{\phi}_2$ which depend on $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_1)$. We term this a test of an ULSS-1 structure for global *a priori* identifiability under incomplete information.

Drawing on the development in this chapter, we present below a scheme for testing an ULSS-1 structure for global *a priori* identifiability in the Structure Classification Requiring Minimality Information (SCReMI) algorithm. We may use this as an alternative to the SCUII Algorithm.

SCReMI Algorithm. Given an ULSS-1 structure M:

1. Use M to define uncontrolled LTI structures $M^{[1]}$ and $M^{[2]}$ following Section 4.3.1. Determine whether it is reasonable to assume that the conditions of Definition 4.1

hold. If so, proceed to Step 2.

2. Determine $\boldsymbol{\phi}_1$ from $M^{[1]}(\boldsymbol{\theta_1})$ and calculate $\mathcal{I}(M^{[1]}, \boldsymbol{\phi}_1)$ (see Definition 3.19). As we know that $\mathcal{I}(M, \boldsymbol{\phi}) \subseteq \mathcal{I}(M^{[1]}, \boldsymbol{\phi}_1)$, if $|\mathcal{I}(M^{[1]}, \boldsymbol{\phi}_1)| = 1$ then M is globally *a priori* identifiable and there is no need to proceed further. If $|\mathcal{I}(M^{[1]}, \boldsymbol{\phi}_1)| > 1$ and the result of the test is not acceptable, response invariants of $M^{[2]}(\boldsymbol{\theta})$ are required. Proceed to Step 3.

3. Test $M^{[2]}$ for generic minimality. If $M^{[2]}$ is not generically minimal, no further classification of M is possible with this algorithm. If $M^{[2]}$ is generically minimal, the unprocessed form of $\mathcal{L}\{y^{[2]}\}(s)$ is the canonical form. The coefficients of s in the denominator are invariants. Collect these into a vector $\boldsymbol{\phi}_s$, a subvector of $\boldsymbol{\phi}_2$. Determine $\grave{\boldsymbol{\phi}} \triangleq \langle \boldsymbol{\phi}_1, \boldsymbol{\phi}_s \rangle$. Proceed to Step 4.

4. Determine $\mathcal{I}(M, \grave{\boldsymbol{\phi}})$. If $|\mathcal{I}(M, \grave{\boldsymbol{\phi}})| = 1$ then M is globally *a priori* identifiable. Otherwise, as $\mathcal{I}(M, \boldsymbol{\phi}) \subseteq \mathcal{I}(M, \grave{\boldsymbol{\phi}})$, $|\mathcal{I}(M, \grave{\boldsymbol{\phi}})|$ may overestimate the number of elements in $\mathcal{I}(M, \boldsymbol{\phi})$.

   If the number of elements in $\mathcal{I}(M, \grave{\boldsymbol{\phi}})$ is greater than one and at most countably infinite, then M is at worst locally *a priori* identifiable.

   If the number of elements in $\mathcal{I}(M, \grave{\boldsymbol{\phi}})$ is uncountably infinite, then it is not possible to classify M with this algorithm.

   Unless M is globally *a priori* identifiable, we cannot exactly classify M with this algorithm as we are unable to use those elements of $\boldsymbol{\phi}_2$ that appear in the numerator of $\mathcal{L}\{y^{[2]}\}$.

4.7 Prelude to Chapter 5

In this chapter we proposed approaches to testing a structure of uncontrolled linear switching systems of one switching event for global *a priori* identifiability. These build on elements of the theory used for LTI structures. This process has highlighted some potential difficulties in the application of the theory of global *a priori* identifiability to a ULSS-1

structure. We have advanced some approaches to these issues. We will demonstrate the utility of the two algorithms proposed for testing a ULSS-1 structure for global *a priori* identifiability by applying them to test cases in Chapter 5.

These test cases are structures representing interaction models seen in the biosensor literature. In particular, these are the 'simple bimolecular interaction' (formalised in Whyte [99]) and the 'two-state conformational change model' (Whyte [97]).

In our exploration of mathematical representations of the simple bimolecular model, we derived an 'initial structure' that had four parameters. From this we derived a 'revised structure' which had three parameters. We may obtain a practical benefit when a revised structure has fewer parameters than the initial structure. That is, we may expect that a process for estimating parameters of a structure is completed more quickly for the revised structure than it is for the initial structure. The advantage of the revised structure is even greater when it is globally *a priori* identifiable whilst the initial structure is *a priori* unidentifiable. We will observe this result as we scrutinise the four- and three-parameter forms of the simple bimolecular model shortly.

The two forms of the simple bimolecular model are classified in a reasonably straightforward manner. The two-state conformational change model provides a more challenging test case for the theory we advanced in this chapter.

Chapter 5

Application of the mathematical theory to test cases

5.1 Overview

In this chapter, we apply methods for testing a ULSS-1 structure for global *a priori* identifiability proposed in Chapter 4 to test cases formally specified in Whyte [97, 99]. These are the four-parameter version of the simple bimolecular interaction, the three-parameter version of the same, and the two-state conformational change model.

For the first two test cases, when each ULSS-1 structure is used to define component LTI structures, these are clearly generically minimal. As a result, it is not necessary to apply the SCUII Algorithm. We cannot use the SCReMI Algorithm to classify the four-parameter form without a supplementary consideration of its response function. We will see that two parameters only occur as a product, leading to a judgement of the structure as *a priori* unidentifiable. However, we can readily use the SCReMI Algorithm to classify the three-parameter version — a reparameterisation of the four-parameter form — as globally *a priori* identifiable. The results indicate that our methods for testing a structure are able to anticipate when it is unfit for parameter estimation. They also show that an

unfavourable classification can indicate how to reparameterise the structure to yield an alternative that is suitable.

The two-state conformational change model provides a more challenging test of the proposed global *a priori* identifiability theory. We apply the SCUII Algorithm first, and we will see that it is able to infer that the ULSS-1 structure representing the interaction is globally *a priori* identifiable. Application of the SCReMI Algorithm to this test case is more laborious due to the need to determine whether or not the structure defined by the second subsystem of the representative ULSS-1 is generically minimal. Ultimately this is decided in the affirmative. This leads to the conclusive classification of the structure as globally *a priori* identifiable, reproducing the result obtained by the SCUII Algorithm. This agreement suggests the potential usefulness of the SCUII Algorithm as a relatively easy-to-apply method of classifying structures representing biomolecular interactions in kinetic experiments.

We will examine the test cases shortly. First, let us recall the discussion of the mismatch of data and structure output presented in Section 3.6.4.2. We will draw on this in the following subsection. There we will consider conditions under which the result of testing a structure for global *a priori* identifiability is not an accurate indication of the likely worth of planned experiments.

5.1.1 Sources of constant response from flow-cell biosensor experiments or assumed structures

Recall that Assumption 3.1 described the expectation that idealised data obtained from a structure for use in testing that structure for global *a priori* identifiability is a true idealisation of experimental data. We noted that if this assumption does not hold, that is, if there is a structure-data mismatch, this may invalidate the result of the test.

We will consider the issue of structure-data mismatch for data from a kinetic experiment of two phases and idealised data from our assumed ULSS-1 structures. We will

consider those values of experimental variables or parameters for which idealised data is constant for the entire duration of at least one experimental phase.

Let us first consider features of the class of model structures we use to model kinetic experiments. Consider some ULSS-1 structure M. If we wish to test M for global *a priori* identifiability using either the SCUII Algorithm or the SCReMI Algorithm, we must derive uncontrolled LTI structures $M^{[1]}$ and $M^{[2]}$ from M. For the kinetic experiments we consider, $M^{[1]}$ represents the response and dynamics of the association phase. In an analogous manner, $M^{[2]}$ summarises the dissociation phase. Recall that these structures have state vectors $\mathbf{x}^{[\mathbf{1}]}$ and $\mathbf{x}^{[\mathbf{2}]}$, and responses $y^{[1]}$ and $y^{[2]}$, respectively. Following Example 3.1, if $\mathbf{x}^{[\mathbf{1}]}$ or $\mathbf{x}^{[\mathbf{2}]}$ is constant for all time, so is the response of the structure which generates it. The test of a ULSS-1 structure for global *a priori* identifiability given in Definition 4.1 anticipates this case.

However, Assumption 3.1 is also violated for other conditions that cause a kinetic experiment to produce data that takes a constant value for at least one experimental phase. This occurs for the experimental conditions summarised in Table 5.1.1. We will label experiments performed under these conditions as "uninformative experiments". The table also shows whether it is possible to anticipate these prior to conducting experiments, and how conditions should be changed to produce more useful data. We see that knowledge of the experimental system makes it possible to avoid constant response due to the tabulated conditions.

Table 5.1.1: Causes of constant experimental response in either a phase of a kinetic experiment or from a model structure assumed for this system.

Category	**Cause of constant state (constant binding response)**	**Able to anticipate prior to full experimental series?**	**Remedy?**
experimental features: association phase	no functional immobilised ligand	yes: defines a "control" run (reference cell), or is otherwise seen in a pilot experiment	if not for a control, redo/change immobilisation step
	no analyte in injected solution	yes: only occurs for a "blank" run	if not for a blank, inject solution known to contain analyte
	no complex formation	yes: seen in a pilot experiment	no interaction to study further, or, redesign planned experimental conditions to facilitate binding
	analyte molecular mass below biosensor detection threshold (see Section 2.3)	yes: by knowing apparatus limitations	use Surface Competition Assay (not Direct Binding Assay, see Section 2.4.2)
experimental features: dissociation phase	complex forms in association phase, then does not dissociate	yes: seen in a pilot experiment	use equilibrium (not kinetic) experiment and appropriate structure, or change experimental conditions to promote dissociation
structure features: association and dissociation	parameters/variables representing analyte, immobilised ligand or rate constants are zero	yes: values are obviously unphysical for typical experimental conditions	restrict variables and parameters to positive values
	initial state of phase is an equilibrium state	yes: conditions of identifiability test reveal regions of parameter space where this occurs for the association phase (not for dissociation phase as long as complex forms previously)	no action required for atypical behaviour

Insights gained from this inspection direct us to analogous causes of a model structure producing constant response. These are shown in the lower part of Table 5.1.1. From this we see that one source of a constant state is when values of variables correspond to the conditions of uninformative experiments shown in the upper portion of the table. A constant state could also occur if parameters representing rate constants were zero, indicating that an interaction cannot occur. However, by convention, a rate constant has a positive value. Restricting parameters to positive values is also a consequence of knowing from pilot experiments that an interaction does actually occur. Taking this action allows us to dismiss as infeasible various situations under which a structure could produce constant response for at least one phase of an experiment.

Let us summarise the discussion of this subsection. We considered situations under which Assumption 3.1 is violated for the type of ULSS-1 structure we employ to model kinetic experiments. For structures and experiments subject to typical experimental conditions, Table 5.1.1 indicates that we can disregard many situations which violate Assumption 3.1 as they are implausible. However, it is necessary to ensure that a ULSS-1 structure satisfies the conditions of Definition 4.1 prior to testing it as we cannot decide if these are satisfied merely by inspection. Should a structure satisfy these conditions, testing a ULSS-1 structure is a valid means of judging the value of proposed experiments.

We now proceed to consider the first of our test cases.

5.2 Test case $\mathcal{M}$: the three-parameter simple bimolecular reaction model

The 'simple bimolecular model' considers the interaction between free analyte (A) and free immobilised ligand (B) in a flow-cell optical biosensor experiment. Formation and breakdown of complex AB is represented by the chemical equation

$$\mathrm{A} + \mathrm{B} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{AB}, \tag{5.2.1}$$

where k_a (k_d) is the rate constant of the forward (reverse) reaction. This interaction is widely assumed in the analysis of flow-cell biosensor data. Whyte [99] reviewed representations of this interaction. We proposed structures of uncontrolled parametric linear switching systems of one switching event for the interaction of species and resultant response in Whyte [90, 94]. We will use these shortly.

Let us use $\mathcal{M}$ to denote the model structure. The representative system of $\mathcal{M}$, System $\mathcal{M}(\boldsymbol{\theta})$, has response $y(\cdot, \boldsymbol{\theta})$ and is of the form of (3.7.41) with state vector $\mathbf{x}$ representing scaled values of [B] (having initial value β_1) and [AB], $X = \mathbb{R}^2_+$, $Y = \mathbb{R}_+$, analyte concentration α_1, and

$$\begin{gathered}
\boldsymbol{\theta_1} = (k_a, k_d, \beta_1)^\top \in \mathbb{R}^3_+, \quad \boldsymbol{\theta_2} = (k_d)^\top \in \mathbb{R}^1_+, \\
\boldsymbol{\theta} = \boldsymbol{\theta}_1 \in \Theta = \mathbb{R}^3_+, \quad \alpha_1 > 0, \\
\mathbf{A_1}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 & k_d \\ k_a\alpha_1 & -k_d \end{bmatrix}, \quad \mathbf{A_2}(\boldsymbol{\theta_2}) = \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix}, \\
\mathbf{C_1} = \mathbf{C_2} = \begin{bmatrix} 0 & 1 \end{bmatrix}, \qquad \mathbf{x_0}(\boldsymbol{\theta_1}) = \begin{bmatrix} \beta_1 & 0 \end{bmatrix}^\top.
\end{gathered} \tag{5.2.2}$$

System (5.2.2) defines a structure of three parameters. Testing $\mathcal{M}$ for global *a priori* identifiability following the SCReMI Algorithm requires definition of LTI structures $\mathcal{M}^{[1]}$ and $\mathcal{M}^{[2]}$ as described in Section 4.3.1. We present the Maple worksheet used in the following analysis of $\mathcal{M}$ in Appendix C.

5.2.1 Consideration of LTI $\mathcal{M}^{[1]}$ obtained from $\mathcal{M}$

The first consideration for $\mathcal{M}^{[1]}$ (having representative system $\mathcal{M}^{[1]}(\boldsymbol{\theta})$ and state function $\mathbf{x}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta})$) is the condition of Definition 4.1 that for almost all $\boldsymbol{\theta} \in \Theta$, $\dot{\mathbf{x}}^{[\mathbf{1}]}(t, \boldsymbol{\theta}) \neq \mathbf{0}\ \forall t \in \bar{\mathbb{R}}_+$.

Following the discussion of Section 5.1, we require the amount of functional immobilised ligand and concentration of injected analyte to have positive values. That is, $\beta_1 > 0$

and $\alpha_1 > 0$ in (5.2.2). Further, Definition 4.1 requires that $\mathcal{M}^{[1]}(\boldsymbol{\theta})$ has

$$\dot{\mathbf{x}}^{[\mathbf{1}]}(0,\boldsymbol{\theta}) = \mathbf{A_1}(\boldsymbol{\theta})\mathbf{x_0}(\boldsymbol{\theta}) = \begin{bmatrix} -k_a\alpha_1\beta_1 \\ k_a\alpha_1\beta_1 \end{bmatrix} \neq \mathbf{0}. \tag{5.2.3}$$

As α_1 and β_1 are positive, and k_a is also positive (by convention, recall Table 5.1.1), (5.2.3) is satisfied for all feasible parameters and experimental conditions.

The unprocessed Laplace transform of response of $\mathcal{M}^{[1]}(\boldsymbol{\theta})$ gives

$$\begin{aligned} \mathcal{L}\{y^{[1]}(\cdot,\boldsymbol{\theta})\}(s) &= \frac{\varphi_2^{[1]}(\boldsymbol{\theta})}{s^2 + \varphi_1^{[1]}(\boldsymbol{\theta})s + \varphi_0^{[1]}(\boldsymbol{\theta})}, \quad \forall s \in \mathrm{H}_0, \\ \varphi_0^{[1]} &\equiv 0, \quad \varphi_1^{[1]}(\boldsymbol{\theta}) = k_a\alpha_1 + k_d, \quad \varphi_2^{[1]}(\boldsymbol{\theta}) = k_a\alpha_1\beta_1. \end{aligned} \tag{5.2.4}$$

As the numerator of $\mathcal{L}\{y^{[1]}(\cdot,\boldsymbol{\theta})\}(s)$ in (5.2.4) is constant, there are no factors common to the numerator and denominator and hence $\mathcal{M}^{[1]}$ is generically minimal. As the denominator of $\mathcal{L}\{y^{[1]}(\cdot,\boldsymbol{\theta})\}(s)$ is also monic, (5.2.4) is in the canonical form (recall Definition 3.26) and hence $\phi_i^{[1]} \equiv \varphi_i^{[1]}$ for $i = 0, 1, 2$.

In considering output of $\mathcal{M}^{[1]}(\boldsymbol{\theta})$, $\boldsymbol{\theta} \in \mathbb{R}^3_+$ and $\alpha_1 > 0$, hence $\phi_1^{[1]}(\boldsymbol{\theta_1})$ and $\phi_2^{[1]}(\boldsymbol{\theta_1})$ are strictly non-zero $\forall \boldsymbol{\theta} \in \Theta$. As such, we can determine them from $y^{[1]}$, as is expected from the discussion of Section 5.1.

Denote the vector of (non-identically zero) moment invariants obtainable from the response of $\mathcal{M}^{[1]}(\boldsymbol{\theta})$ by $\boldsymbol{\phi}_1(\boldsymbol{\theta}) \triangleq \left(\phi_1^{[1]}(\boldsymbol{\theta}), \phi_2^{[1]}(\boldsymbol{\theta})\right)^\top$, where the components are shown in (5.2.4). Step 2 of the SCReMI Algorithm requires determination of $\mathcal{I}(\mathcal{M}, \boldsymbol{\phi}_1)$. This is a set of solutions of two equations

$$\begin{aligned} k_a\alpha_1 + k_d &= k'_a\alpha_1 + k'_d\,, \\ k_a\alpha_1\beta_1 &= k'_a\alpha_1\beta'_1\,, \end{aligned}$$

in three unknowns (as we assume that α_1 is known). Hence, the elements of $\mathcal{I}(\mathcal{M}, \boldsymbol{\phi}_1)$ are non-denumerable. As a result, we can make no useful judgement on $\mathcal{M}$ using only the information obtained by observing the system before the switching event. Hence, the test of $\mathcal{M}$ for global *a priori* identifiability requires consideration of $\mathcal{M}^{[2]}$.

5.2.2 Consideration of LTI $\mathcal{M}^{[2]}$ obtained from $\mathcal{M}$

The condition imposed by Definition 4.1 on $\mathcal{M}^{[2]}$ (having representative system $\mathcal{M}^{[2]}(\boldsymbol{\theta})$ and state function $\mathbf{x}^{[2]}(\cdot,\boldsymbol{\theta})$) requires that for almost all $\boldsymbol{\theta} \in \Theta$, $\dot{\mathbf{x}}^{[2]}(t,\boldsymbol{\theta}) \neq \mathbf{0}\ \forall t \in \bar{\mathbb{R}}_+$. We summarise this condition by

$$\dot{\mathbf{x}}^{[2]}(0,\boldsymbol{\theta}) = \mathbf{A_2}(\boldsymbol{\theta})\mathbf{x}^{[1]}(t_1,\boldsymbol{\theta}) = \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix} \mathbf{x}^{[1]}(t_1,\boldsymbol{\theta}) \neq \mathbf{0}. \tag{5.2.5}$$

Let us represent the initial state of $\mathcal{M}^{[2]}(\boldsymbol{\theta})$ as $\mathbf{x}^{[1]}(t_1,\boldsymbol{\theta}) \triangleq (x_1^{[1]}(t_1,\boldsymbol{\theta}), x_2^{[1]}(t_1,\boldsymbol{\theta}))^\top$. Condition (5.2.5) is satisfied whenever $k_d \cdot x_2^{[1]}(t_1,\boldsymbol{\theta}) \neq 0$. This holds for any typical association phase as $k_d > 0$ and when complex forms $x_2^{[1]}(t_1,\boldsymbol{\theta}) > 0$.

The unprocessed form of the Laplace transform of the output of $\mathcal{M}_2(\boldsymbol{\theta})$ is

$$\begin{gathered} \mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s) = \frac{\varphi_1^{[2]}(\boldsymbol{\theta})}{s+\varphi_0^{[2]}(\boldsymbol{\theta})}, \quad \forall s \in \mathrm{H}_{-k_d}, \\ \varphi_0^{[2]}(\boldsymbol{\theta}) = k_d, \quad \varphi_1^{[2]}(\boldsymbol{\theta}) = x_2^{[1]}(t_1,\boldsymbol{\theta_1}). \end{gathered} \tag{5.2.6}$$

As we saw for $\mathcal{L}\{y^{[1]}(\cdot,\boldsymbol{\theta_1})\}(s)$, $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta_1})\}(s)$ is also of the canonical form; pole-zero cancellation cannot occur and hence $\mathcal{M}^{[2]}$ is generically minimal. Hence, we obtain response invariants directly from (5.2.6) by $\phi_i^{[2]} \equiv \varphi_i^{[2]}$ for $i = 0, 1$. We represent the collection of these by $\boldsymbol{\phi}_2(\boldsymbol{\theta}) \triangleq \left(\phi_0^{[2]}(\boldsymbol{\theta}), \phi_1^{[2]}(\boldsymbol{\theta})\right)^\top$. These are non-zero by positivity of both the parameter vector and $x_2^{[1]}(t_1,\boldsymbol{\theta_1})$ (as discussed in Section 5.1).

5.2.3 Classification of $\mathcal{M}$ using incomplete information from $\mathcal{M}^{[2]}$

The usual manner of testing a (non-switching) structure for global *a priori* identifiability is to use all response invariants $\boldsymbol{\phi}(\boldsymbol{\theta})$ in the test. However, our aim here is to ascertain if we can decide that ULSS-1 structure $\mathcal{M}$ is globally *a priori* identifiable without obtaining $\boldsymbol{\phi}(\boldsymbol{\theta})$. In particular, we wish to avoid obtaining an explicit expression for the initial conditions of $\mathcal{M}^{[2]}(\boldsymbol{\theta})$ which appear in certain invariants (see Section 4.6). Rather than use $\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq \left(\boldsymbol{\phi}_1(\boldsymbol{\theta})^\top, \boldsymbol{\phi}_2(\boldsymbol{\theta})^\top\right)^\top$ as per usual, Step 3 of the SCReMI Algorithm provides

an alternative. Consider the subvector of $\boldsymbol{\phi}(\boldsymbol{\theta})$ given by $\grave{\boldsymbol{\phi}}(\boldsymbol{\theta}) \triangleq (\boldsymbol{\phi}_{\mathbf{1}}(\boldsymbol{\theta})^\top, \phi_0^{[2]}(\boldsymbol{\theta}))^\top$. We draw the elements of this vector from the canonical form of Laplace transforms of response given by (5.2.4) and (5.2.6).

Using $\grave{\boldsymbol{\phi}}$ in Step 4 of the SCReMI Algorithm shows $\mathcal{I}(\mathcal{M}, \grave{\boldsymbol{\phi}}) = \{\boldsymbol{\theta}\}$. Hence we have shown that $\mathcal{M}$ is globally *a priori* identifiable. We have achieved this in the absence of an explicit expression for $\phi_1^{[2]}$, as would be required for the calculation of $\mathcal{I}(\mathcal{M}, \boldsymbol{\phi})$. This suggests that $\phi_1^{[2]}$ does not contain any information on the parameters beyond that available in $\grave{\boldsymbol{\phi}}$. Having an understanding of this result may assist in justifying the use of $\mathcal{I}(\mathcal{M}, \grave{\boldsymbol{\phi}})$ rather than $\mathcal{I}(\mathcal{M}, \boldsymbol{\phi})$ for other test cases. Note that in (5.2.6) $\phi_1^{[2]}(\boldsymbol{\theta}) \triangleq x_2^{[1]}(t_1, \boldsymbol{\theta}) \equiv y^{[1]}(t_1, \boldsymbol{\theta})$. Thus, knowledge of $\phi_1^{[2]}(\boldsymbol{\theta})$ is contained within knowledge of $y^{[1]}(t, \boldsymbol{\theta})$ $\forall t \in [0, t_1)$, that is, the observations of the system representing the association phase of an experiment. Relation (5.2.4) shows that $y^{[1]}(t, \boldsymbol{\theta})$ is a function of $\phi_1^{[1]}$ and $\phi_2^{[1]}$. Hence, $\phi_1^{[2]}$ provides no additional information on $\mathcal{M}(\boldsymbol{\theta})$ beyond that included in $\boldsymbol{\phi}_1$. The redundancy of $\phi_1^{[2]}$ in the process of testing $\mathcal{M}$ for global *a priori* identifiability follows accordingly.

The four-parameter version of the simple bimolecular model (see Whyte [99]) provides another test case for the process of testing a ULSS-1 structure for global *a priori* identifiability described by the SCReMI Algorithm. This test case demonstrates the influence of the parameterisation used in the model structure on the result of the test.

5.3 Test case $\mathcal{N}$: the four-parameter simple bimolecular model

Let the ULSS-1 structure be denoted by $\mathcal{N}$ with output $\hat{y}$. Its representative system $\mathcal{N}(\boldsymbol{\theta})$ is of the form of Equation (3.7.41) with $X = \mathbb{R}^2_+$, $Y = \bar{\mathbb{R}}_+$, and

$$\begin{gathered}
\boldsymbol{\theta_1} = (k_a, k_d, B_0, \rho_A)^\top \in \mathbb{R}^4_+, \quad \boldsymbol{\theta_2} = (k_d, \rho_A)^\top \in \mathbb{R}^2_+, \\
\boldsymbol{\theta} = \boldsymbol{\theta}_1 \in \Theta = \mathbb{R}^4_+, \quad \alpha_1 > 0, \\
\mathbf{A_1}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 & k_d \\ k_a\alpha_1 & -k_d \end{bmatrix}, \quad \mathbf{A_2}(\boldsymbol{\theta_2}) = \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix}, \\
\mathbf{C_1} = \mathbf{C_2} = \begin{bmatrix} 0 & \rho_A \end{bmatrix}, \qquad \mathbf{x_0}(\boldsymbol{\theta_1}) = \begin{bmatrix} B_0 & 0 \end{bmatrix}^\top.
\end{gathered} \tag{5.3.7}$$

Testing $\mathcal{N}$ according to the SCReMI Algorithm requires us to define the LTI structures describing the dynamics of the association and dissociation phases of an experiment. Here these are $\mathcal{N}^{[1]}$ (output $\hat{y}^{[1]}$) and $\mathcal{N}^{[2]}$ (output $\hat{y}^{[2]}$) respectively. We present the Maple worksheet used in the following analysis in Appendix D.

5.3.1 Consideration of LTI $\mathcal{N}^{[1]}$ obtained from $\mathcal{N}$

Let us consider the condition of Definition 4.1 applied to $\mathcal{N}^{[1]}$ (having representative system $\mathcal{N}^{[1]}(\boldsymbol{\theta})$ and state function $\mathbf{x}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta})$) that for almost all $\boldsymbol{\theta} \in \Theta$, $\dot{\mathbf{x}}^{[\mathbf{1}]}(t, \boldsymbol{\theta}) \neq \mathbf{0}$ $\forall t \in \bar{\mathbb{R}}_+$. That is, $\mathcal{N}^{[1]}(\boldsymbol{\theta_1})$ is subject to

$$\dot{\mathbf{x}}^{[1]}(0, \boldsymbol{\theta_1}) = \mathbf{A_1}(\boldsymbol{\theta_1})\mathbf{x_0}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 B_0 \\ k_a\alpha_1 B_0 \end{bmatrix} \neq \mathbf{0}. \tag{5.3.8}$$

Our argument here is very similar to that applied to $\mathcal{M}^{[1]}$ in Section 5.2.1. Following the discussion of Section 5.1, the need for positive values for the amount of functional immobilised ligand and concentration of injected analyte result in conditions $B_0 > 0$ and $\alpha_1 > 0$. As k_a is positive by convention, (5.3.8) is satisfied for all feasible combinations of parameter values and variables representing experimental conditions.

Proceeding to step 2 of the SCReMI Algorithm, one sees that the unprocessed form of $\mathcal{L}\{\hat{y}^{[1]}(\cdot,\boldsymbol{\theta})\}(s)$ is the canonical form, given by

$$\begin{gathered}\mathcal{L}\{\hat{y}^{[1]}(\cdot,\boldsymbol{\theta})\}(s)=\frac{\phi_2^{[1]}(\boldsymbol{\theta})}{s^2+\phi_1^{[1]}(\boldsymbol{\theta})s+\phi_0^{[1]}(\boldsymbol{\theta})},\quad s\in \mathrm{H}_0,\\ \phi_0^{[1]}(\boldsymbol{\theta})\equiv 0,\quad \phi_1^{[1]}(\boldsymbol{\theta})=k_a\alpha_1+k_d,\quad \phi_2^{[1]}(\boldsymbol{\theta})=k_a\alpha_1\rho_A B_0.\end{gathered}\tag{5.3.9}$$

We see that $\phi_1^{[1]}(\boldsymbol{\theta})$ and $\phi_2^{[1]}(\boldsymbol{\theta})$ in (5.3.9) are non-zero as $\boldsymbol{\theta}\in\mathbb{R}_+^4$ and $\alpha_1>0$. Hence, the collection of moment invariants from $\mathcal{N}^{[1]}$ is

$$\boldsymbol{\phi}_1(\boldsymbol{\theta})\triangleq\left(\phi_1^{[1]}(\boldsymbol{\theta}),\phi_2^{[1]}(\boldsymbol{\theta})\right)^\top.\tag{5.3.10}$$

We test $\mathcal{N}$ for global *a priori* identifiability using only the parameter information present in response prior to the switching event ($\boldsymbol{\phi}_1$ defined by (5.3.10)) by determining $\mathcal{I}(\mathcal{N},\boldsymbol{\phi}_1)$. Here,

$$\begin{aligned}&\mathcal{I}(\mathcal{N},\boldsymbol{\phi}_1)=\\ &\qquad\left\{\boldsymbol{\theta}'\in\mathbb{R}_+^4 : k_a'=\frac{\rho_A B_0 k_a}{\rho_A' B_0'},\ k_d'=\frac{(k_a\alpha_1+k_d)\rho_A' B_0'-k_a\alpha_1\rho_A B_0}{\rho_A' B_0'}\right\},\end{aligned}\tag{5.3.11}$$

where B_0' and ρ_A' are free to take any positive value.

Inspection of (5.3.11) shows that the elements of $\mathcal{I}(\mathcal{N},\boldsymbol{\phi}_1)$ are non-denumerable. This is to be expected as $\mathcal{I}(\mathcal{N},\boldsymbol{\phi}_1)$ is the solution set of two equations in four parameters. Further, $\mathcal{I}(\mathcal{N},\boldsymbol{\phi}_1)$ does not obviously show that any of the parameters are either globally or locally *a priori* identifiable. As (5.3.11) does not permit a useful judgement on $\mathcal{N}$ or its parameters, we proceed with the SCReMI Algorithm and consider the response of $\mathcal{N}^{[2]}$.

5.3.2 Consideration of LTI $\mathcal{N}^{[2]}$ obtained from $\mathcal{N}$

The initial state of $\mathcal{N}^{[2]}(\boldsymbol{\theta})$, $\mathbf{x}^{[2]}(0,\boldsymbol{\theta}_1)$, is $\mathbf{x}^{[1]}(t_1,\boldsymbol{\theta}_1)\triangleq(x_1^{[1]}(t_1,\boldsymbol{\theta}_1),x_2^{[1]}(t_1,\boldsymbol{\theta}_1))^\top$. The condition imposed by Definition 4.1 on $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ is similar to that imposed on $\mathcal{M}^{[2]}(\boldsymbol{\theta})$ in (5.2.5), and it is similarly satisfied for all feasible parameter values.

Determination of the unprocessed form of the Laplace transform of $\hat{y}^{[2]}(\cdot, \boldsymbol{\theta})$ shows that it is also in the canonical form:

$$\begin{gathered} \mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s) = \frac{\phi_1^{[2]}(\boldsymbol{\theta})}{s + \phi_0^{[2]}(\boldsymbol{\theta}_2)}, \quad s \in \mathrm{H}_{-k_d}, \\ \phi_0^{[2]}(\boldsymbol{\theta}_2) = k_d, \quad \phi_1^{[2]}(\boldsymbol{\theta}) = \rho_A x_2^{[1]}(t_1, \boldsymbol{\theta}_1). \end{gathered} \tag{5.3.12}$$

Recalling the discussion of Section 5.1, the moment invariants in (5.3.12) are non-zero. Hence, (5.3.12) supplies the invariants of $\mathcal{M}^{[2]}(\boldsymbol{\theta})$:

$$\boldsymbol{\phi}_2(\boldsymbol{\theta}) \triangleq \left(\phi_0^{[2]}(\boldsymbol{\theta}_2), \phi_1^{[2]}(\boldsymbol{\theta})\right)^\top.$$

5.3.3 An (unsuccessful) attempt to classify $\mathcal{N}$ using incomplete $\mathcal{N}^{[2]}$ information

Following Step 3 of the SCReMI Algorithm, we defer determining an expression for $x_2^{[1]}(t_1, \boldsymbol{\theta}_1)$ by defining $\grave{\boldsymbol{\phi}}(\boldsymbol{\theta}) = (\boldsymbol{\phi}_1(\boldsymbol{\theta})^\top, \phi_0^{[2]}(\boldsymbol{\theta}))^\top$. This is a subvector of the full set of invariants we obtain by excluding $\phi_1^{[2]}(\boldsymbol{\theta})$ from $\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq (\boldsymbol{\phi}_1(\boldsymbol{\theta})^\top, \boldsymbol{\phi}_2(\boldsymbol{\theta})^\top)^\top$. For this $\grave{\boldsymbol{\phi}}(\boldsymbol{\theta})$,

$$\mathcal{I}(\mathcal{N}, \grave{\boldsymbol{\phi}}) = \left\{ \boldsymbol{\theta}' \in \mathbb{R}_+^4 : k_a' = k_a,\ k_d' = k_d,\ B_0' = \frac{\rho_A B_0}{\rho_A'} \right\}. \tag{5.3.13}$$

The set (5.3.13) shows that k_a and k_d are globally *a priori* parametrically identifiable (recall Definition 3.18). This solution set also shows $\rho_A' B_0' = \rho_A B_0$, suggesting that ρ_A and B_0 may be *a priori* parametrically unidentifiable. This is because for any real $c > 0$, $\rho_A' = \rho_A / c$ and $B_0' = c B_0$ are physically sensible values that satisfy the relation. The result of the test is unsatisfactory as it is unable to either show that $\mathcal{N}$ is globally *a priori* identifiable, or demonstrate that it cannot be *a priori* unidentifiable.

5.3.4 Supplementary use of the state vector of $\mathcal{N}^{[1]}$ in classifying $\mathcal{N}$

As the attempt to classify $\mathcal{N}$ using $\mathcal{I}(\mathcal{N}, \grave{\boldsymbol{\phi}})$ was not conclusive, it is tempting to return to a more conventional approach and determine $\mathcal{I}(\mathcal{N}, \boldsymbol{\phi})$. This requires obtaining an explicit expression for $x_2^{[1]}(t_1, \boldsymbol{\theta}_1)$ to allow the formation of $\phi_1^{[2]}(\boldsymbol{\theta})$ in (5.3.12), and the completion

of $\boldsymbol{\phi}$. Pursuing this approach will require the resolution of certain difficulties. Invariants with a dependence on the fixed but arbitrary time (here, t_1) are not accommodated by the standard theory and testing processes. Also, a complication noted in Remark 4.4 is applicable when invariants are used to form the equations at the core of the identifiability test, as in (3.5.20). There is also the matter of how to combine the solutions of the equation featuring t_1 with the others, and interpret the results.

Rather than becoming enmeshed in these difficulties, let us recall the study of $\mathcal{M}$ in Section 5.2.3. There we found that $\boldsymbol{\phi}(\boldsymbol{\theta})$ did not provide any restriction on parameter values $\boldsymbol{\theta}$ beyond those given by the readily-obtainable $\grave{\boldsymbol{\phi}}(\boldsymbol{\theta})$. Should this property hold in the current setting, we would not need to determine $\phi_1^{[2]}(\boldsymbol{\theta})$ and we would have $\mathcal{I}(\mathcal{N}, \boldsymbol{\phi}) = \mathcal{I}(\mathcal{N}, \grave{\boldsymbol{\phi}})$. Hence, we would judge $\mathcal{N}$ as *a priori* unidentifiable as a result of (5.3.13). Further, obtaining $\mathcal{I}(\mathcal{N}, \boldsymbol{\phi})$ would not change the judgement informed by inspection of $\mathcal{I}(\mathcal{N}, \grave{\boldsymbol{\phi}})$ that k_a and k_d are globally *a priori* parametrically identifiable.

Let us consider how we might anticipate that obtaining $\phi_1^{[2]}(\boldsymbol{\theta})$ is unnecessary. Consider the form of solution of $\mathbf{x}^{[\mathbf{1}]}$. Recall the solution for the state of an uncontrolled LTI system of n states given in Section 3.6.2.1. Using Equation (3.6.28), we may write the state of $\mathcal{N}(\boldsymbol{\theta})$ for $t < t_1$ (which is exactly $\mathbf{x}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta})$) as

$$\mathbf{x}(t) = e^{\mathbf{A}_1 t}\mathbf{x_0} = \sum_{i=1}^{2} \mathbf{v}_i e^{\lambda_i t}, \tag{5.3.14}$$

which in this case has

$$\mathbf{v}_i = \left(\mathbf{s}^{(i)'} \begin{bmatrix} B_0 \\ 0 \end{bmatrix} \right) \mathbf{s}_i. \tag{5.3.15}$$

In considering $\hat{y}$ for $t < t_1$, as $\mathbf{C} = [0 \ \rho_A]$ then

$$\hat{y}(t) = \mathbf{C} \sum_{i=1}^{2} \mathbf{v}_i e^{\lambda_i t} = \rho_A B_0 \begin{bmatrix} 0 & 1 \end{bmatrix} \sum_{i=1}^{n} s_1^{(i)'} \mathbf{s}_i e^{\lambda_i t}. \tag{5.3.16}$$

As the terms inside the summation are independent of ρ_A and B_0, (5.3.16) shows that these parameters occur only as $\rho_A B_0$. (This type of situation is termed 'inseparability' in Eisenfeld [26].)

Were we to write an expression in the form of (5.3.16) for $\mathbf{x}$ for $t \geq t_1$, we would require a more sophisticated (and cluttered) notation to distinguish between eigenvalues and eigenvectors of $\mathbf{A_1}$ and $\mathbf{A_2}$. However, we can make an observation on the nature of $\mathbf{x}^{[\mathbf{2}]}$ and $\hat{y}^{[2]}$ without this. Note that the denominators of (5.3.9) and (5.3.12) show that the eigenvalues of $\mathbf{A_1}$ and $\mathbf{A_2}$ are distinct. An expression such as (5.3.16) for $\mathbf{x}^{[\mathbf{2}]}(\cdot)$ will feature vectors similar in form to the $\mathbf{v}_i$ in (5.3.15) with $\mathbf{x_0}$ replaced by $\mathbf{x}^{[\mathbf{1}]}(t_1)$, a sum of exponentials as shown in (5.3.14). On forming the expression $\hat{y}^{[2]}(t) = \mathbf{C}\mathbf{x}^{[\mathbf{2}]}(t)$, we find it has a linear dependence on $[B_0\ 0]^\top$ through $\mathbf{x}^{[\mathbf{1}]}(t_1)$. This allows us to rearrange its terms to give a premultiplying factor of $\rho_A B_0$ as in (5.3.16).

As ρ_A and B_0 only appear as $\rho_A B_0$, this shows that $\phi_1^{[2]}(\boldsymbol{\theta})$ in (5.3.12) does not provide any conditions on the parameters beyond those given by $\mathcal{I}(\mathcal{N}, \grave{\boldsymbol{\phi}})$ in (5.3.13). This is an essential feature of the model structure; considering output from the structure with variables set to other values (corresponding to the case where data is collected under additional experimental conditions) to obtain more invariants will not resolve the parameter inseparability. Structure $\mathcal{N}$ is *a priori* unidentifiable, and obtaining an explicit expression for $\mathbf{x}^{[\mathbf{1}]}$ is of no benefit to the test of $\mathcal{N}$ for global *a priori* identifiability.

Although the result of the test is unsatisfactory, gaining awareness of an inseparable parameter combination is useful. This information guided the reparameterisation of the (*a priori* unidentifiable) four-parameter version of the simple bimolecular model into the three-parameter version. We showed that this latter form was globally *a priori* identifiable in Section 5.2. This demonstrates the influence of the choice of structure on the result of an inverse problem involving optical biosensor data.

In the two test cases considered to this point, each structure was classified without needing to obtain the entire set of moment invariants present in the output of the structure's representative system. However, we have not yet needed to test the second LTI structure obtained from a ULSS-1 structure for generic minimality. We explore this matter in the final test case to ensure a thorough examination of the methods we proposed in Chapter 4.

5.4 Test case $\mathcal{C}$: the two-state conformational change model

When the simple bimolecular model is unable to provide a satisfactory fit to data, the 'two-state conformational change model' is often used as an alternative. It assumes that analyte A binds to immobilised ligand B to form complex (AB) capable of isomerising to $(AB)^*$, and that these individual steps are reversible. The interaction is summarised by

$$\mathrm{A} + \mathrm{B} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} (\mathrm{AB}) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (\mathrm{AB})^* . \tag{5.4.17}$$

Whyte [97] reviewed alternative descriptions of (5.4.17) and accompanying model structures. This was used to synthesize a detailed structure representing the interaction model and response due to all interactants in a kinetic experiment. In order to reduce the complexity of this initial model, we modified it to suit the case where the response is processed to remove components that do not relate to the progress of the interaction.[1] As a result, we reduced modelled response to the sum of components due to (AB) and $(AB)^*$.

The representative system of the simplified structure was given as a ULSS-1 after Equation (3.7.41) with state vector $\mathbf{x} = ([\mathrm{B}], [(\mathrm{AB})], [(\mathrm{AB})^*])^\top$ with $X = \mathbb{R}^3_+$, output y with $Y = \bar{\mathbb{R}}_+$, and

$$\begin{gathered}
\boldsymbol{\theta_1} = \boldsymbol{\theta} = (\beta_1, k_a, k_d, k_2, k_{-2}) \in \mathbb{R}^5_+, \quad \boldsymbol{\theta_2} = (k_d, k_2, k_{-2}) \in \mathbb{R}^3_+, \quad \alpha_1 > 0, \\
\mathbf{x}(0, \boldsymbol{\theta_1}) = \begin{bmatrix} \beta_1 \\ 0 \\ 0 \end{bmatrix}, \quad \mathbf{A_1}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 & k_d & 0 \\ k_a\alpha_1 & -(k_d + k_2) & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix}, \\
\mathbf{C_1} = \mathbf{C_2} = \begin{bmatrix} 0 & 1 & 1 \end{bmatrix}, \quad \mathbf{A_2}(\boldsymbol{\theta_2}) = \begin{bmatrix} 0 & k_d & 0 \\ 0 & -(k_d + k_2) & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix}.
\end{gathered} \tag{5.4.18}$$

Here we term the ULSS-1 given by (3.7.41) with (5.4.18) $\mathcal{C}(\boldsymbol{\theta})$, and we use it as the representative system of structure $\mathcal{C}$. Testing $\mathcal{C}$ for global *a priori* identifiability using the

[1] Recall the description of response components given on Page 62.

theory described in Chapter 4 requires first applying the approach of Section 4.3.1 to $\mathcal{C}$. This uses $\mathcal{C}$ to generate LTI structures $\mathcal{C}^{[1]}$ and $\mathcal{C}^{[2]}$, each defined on $T = [0, \infty)$. As certain preliminary steps are common to both the SCUII Algorithm and the SCReMI Algorithm, we address these first before applying the two algorithms to $\mathcal{C}$ in turn.

5.4.1 Consideration of LTI $\mathcal{C}^{[1]}$ obtained from $\mathcal{C}$

We present results derived for this section in Section 3 of Appendix E. Recall the condition of Definition 4.1 on $\mathcal{C}^{[1]}$ (having representative system $\mathcal{C}^{[1]}(\boldsymbol{\theta})$ with state function $\mathbf{x}^{[\mathbf{1}]}(\cdot, \boldsymbol{\theta})$) that for almost all $\boldsymbol{\theta} \in \Theta$, $\dot{\mathbf{x}}^{[\mathbf{1}]}(t, \boldsymbol{\theta}) \neq \mathbf{0}$ $\forall t \in \bar{\mathbb{R}}_+$.

This imposes the general conditions that the amount of functional immobilised ligand and concentration of injected analyte are positive, that is, $\beta_1 > 0$ and $\alpha_1 > 0$ in (5.4.18). By the discussion of Section 5.1, we expect that these conditions are satisfied for a typical experiment.

The particular condition we impose on $\mathcal{C}^{[1]}(\boldsymbol{\theta})$ is

$$\dot{\mathbf{x}}^{[1]}(0, \boldsymbol{\theta_1}) \triangleq \mathbf{A_1}(\boldsymbol{\theta_1})\mathbf{x_0}(\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1\beta_1 \\ k_a\alpha_1\beta_1 \\ 0 \end{bmatrix} \neq \mathbf{0}. \tag{5.4.19}$$

As α_1, β_1 are positive by design, and k_a is positive by convention, (5.4.19) is satisfied for all feasible combinations of parameter values and experimental conditions.

The unprocessed Laplace transform of $y^{[1]}(\cdot, \boldsymbol{\theta_1})$ is

$$\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta_1})\}(s) = \frac{\varphi_4^{[1]}(\boldsymbol{\theta_1})s + \varphi_3^{[1]}(\boldsymbol{\theta_1})}{s^3 + \varphi_2^{[1]}(\boldsymbol{\theta_1})s^2 + \varphi_1^{[1]}(\boldsymbol{\theta_1})s + \varphi_0^{[1]}(\boldsymbol{\theta_1})}, \quad \forall s \in \mathrm{H}_{\lambda_1}, \tag{5.4.20}$$

where $\varphi_0^{[1]}(\boldsymbol{\theta_1}) \equiv 0$, λ_1 is the largest zero of the denominator of (5.4.20), and

$$\begin{aligned} \varphi_1^{[1]}(\boldsymbol{\theta_1}) &= k_a\alpha_1(k_2 + k_{-2}) + k_d k_{-2}, & \varphi_2^{[1]}(\boldsymbol{\theta_1}) &= k_a\alpha_1 + k_d + k_2 + k_{-2}, \\ \varphi_3^{[1]}(\boldsymbol{\theta_1}) &= k_a\alpha_1\beta_1(k_2 + k_{-2}), & \varphi_4^{[1]}(\boldsymbol{\theta_1}) &= k_a\alpha_1\beta_1. \end{aligned} \tag{5.4.21}$$

Checking for pole-zero cancellation in (5.4.20) is a standard problem in the analysis of uncontrolled LTI systems. Here we approach this by substituting the zero of the numera-

tor, $s = -(k_2 + k_{-2})$, into the denominator to determine whether the result is zero. This gives $(k_2 + k_{-2})k_d k_2$ which is non-zero as all parameters are positive. Hence, pole-zero cancellation does not occur in (5.4.20). As a result, the canonical form of $\mathcal{L}\{y^{[1]}(\cdot, \boldsymbol{\theta_1})\}(s)$ is the same as the unprocessed form given in (5.4.20). Hence, from (5.4.21):

$$\boldsymbol{\phi}_1(\boldsymbol{\theta_1}) \triangleq \left(\varphi_1^{[1]}(\boldsymbol{\theta_1}),\, \varphi_2^{[1]}(\boldsymbol{\theta_1}),\, \varphi_3^{[1]}(\boldsymbol{\theta_1}),\, \varphi_4^{[1]}(\boldsymbol{\theta_1})\right)^\top. \tag{5.4.22}$$

Let us now proceed to test $\mathcal{C}$ for global *a priori* identifiability using solely the information obtainable from $\mathcal{C}^{[1]}(\boldsymbol{\theta_1})$ given in (5.4.22). The test has the result

$$\mathcal{I}(\mathcal{C}, \boldsymbol{\phi}_1) = \left\{ \boldsymbol{\theta'} \in \mathbb{R}^5_+ \;\middle|\; \begin{array}{l} k'_a = \dfrac{k_a\alpha_1[k_{-2} + k_2 - k'_{-2}] - k_d(k'_{-2} - k_{-2})}{\alpha_1\left(k_{-2} + k_2 - k'_{-2}\right)}, \\ \beta'_1 = \dfrac{\beta_1 k_a \alpha_1\left(k_{-2} + k_2 - k'_{-2}\right)}{k_a\alpha_1[k_{-2} + k_2 - k'_{-2}] - k_d(k'_{-2} - k_{-2})}, \\ k'_d = \dfrac{k_d k_2}{k_{-2} + k_2 - k'_{-2}}, \quad k'_2 = k_{-2} + k_2 - k'_{-2} \end{array} \right\}, \tag{5.4.23}$$

which, by rearrangement, gives

$$\mathcal{I}(\mathcal{C}, \boldsymbol{\phi}_1) = \left\{ \boldsymbol{\theta'} \in \mathbb{R}^5_+ \;\middle|\; k'_a\beta'_1 = k_a\beta_1, \quad k'_d k'_2 = k_d k_2, \quad k'_2 + k'_{-2} = k_{-2} + k_2 \right\}. \tag{5.4.24}$$

Parameters k_a and β_1 are readily judged as *a priori* unidentifiable from (5.4.24), and hence $\mathcal{C}^{[1]}$ is *a priori* unidentifiable. This result does not allow a judgement on $\mathcal{C}$.

Remark 5.1. Note that (5.4.24) suggests $k'_{-2} < k_{-2} + k_2$ to ensure that $k'_2 > 0$.

The inability of $\mathcal{I}(\mathcal{C}, \boldsymbol{\phi}_1)$ to classify $\mathcal{C}$ as globally or locally *a priori* identifiable suggests proceeding further with the SCReMI Algorithm or SCUII Algorithm. This requires consideration of the parameter information obtainable from $\mathcal{C}^{[2]}$.

5.4.2 Consideration of LTI $\mathcal{C}^{[2]}$ obtained from $\mathcal{C}$

In the following, we represent the initial state of $\mathcal{C}^{[2]}(\boldsymbol{\theta})$, $\mathbf{x}^{[2]}(0, \boldsymbol{\theta})$, by $\boldsymbol{\xi} = (\xi_1, \xi_2, \xi_3)^\top$. Consider the condition imposed by Definition 4.1 on $\mathcal{C}^{[2]}$ (having representative system

$\mathcal{C}^{[2]}(\boldsymbol{\theta})$ with state function $\mathbf{x}^{[\mathbf{2}]}(\cdot, \boldsymbol{\theta})$) that for almost all $\boldsymbol{\theta} \in \Theta$, $\dot{\mathbf{x}}^{[\mathbf{2}]}(t, \boldsymbol{\theta}) \neq \mathbf{0}\ \forall t \in \bar{\mathbb{R}}_+$. We summarise this condition by

$$\dot{\mathbf{x}}^{[\mathbf{2}]}(0, \boldsymbol{\theta}) = \mathbf{A_2}(\boldsymbol{\theta})\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta_1}) = \begin{bmatrix} 0 & k_d & 0 \\ 0 & -(k_d + k_2) & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix} \mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta_1}) \neq \mathbf{0}.$$

By positivity of the rate constants, this condition is satisfied whenever $x_2^{[1]}(t_1, \boldsymbol{\theta_1}) \neq 0$. This is true for any typical association phase, as when complex forms $x_2^{[1]}(t_1, \boldsymbol{\theta_1}) > 0$.

The unprocessed Laplace transform of $y^{[2]}(\cdot, \boldsymbol{\theta})$ is given by

$$\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s) = \frac{\varphi_3^{[2]}(\boldsymbol{\theta})s + \varphi_2^{[2]}(\boldsymbol{\theta})}{s^2 + \varphi_1^{[2]}(\boldsymbol{\theta_2})s + \varphi_0^{[2]}(\boldsymbol{\theta_2})}, \quad \forall s \in \mathrm{H}_{\lambda_l(\boldsymbol{\theta_2})}, \tag{5.4.25}$$

where the largest and smallest denominator roots are λ_l and λ_s respectively, and

$$\begin{aligned} \varphi_0^{[2]}(\boldsymbol{\theta_2}) &= k_d k_{-2}, & \varphi_1^{[2]}(\boldsymbol{\theta_2}) &= k_d + k_2 + k_{-2}, \\ \varphi_2^{[2]}(\boldsymbol{\theta}) &= (\xi_2 + \xi_3)(k_2 + k_{-2}) + \xi_3 k_d, & \varphi_3^{[2]}(\boldsymbol{\theta}) &= \xi_2 + \xi_3. \end{aligned} \tag{5.4.26}$$

Unlike test cases $\mathcal{M}$ and $\mathcal{N}$ in which the second LTI system obtained from a ULSS-1 is clearly generically minimal, it is not at all clear that $\mathcal{C}^{[2]}$ should have this property from inspection of (5.4.25) and (5.4.26). As a result, continuing with the application of the SCReMI Algorithm requires testing $\mathcal{C}^{[2]}$ for generic minimality. We will return to this task after applying the SCUII Algorithm to $\mathcal{C}$.

5.4.3 Application of the SCUII Algorithm to $\mathcal{C}$

This section draws on and expands the analysis first presented in Whyte [94]. We present relevant calculations in Section 9 of Appendix E.

Factorising the denominator of (5.4.25) leads to

$$\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s) = \frac{\varphi_3^{[2]}(\boldsymbol{\theta})s + \varphi_2^{[2]}(\boldsymbol{\theta})}{\big(s - \lambda_l(\boldsymbol{\theta_2})\big)\big(s - \lambda_s(\boldsymbol{\theta_2})\big)}, \tag{5.4.27}$$

where

$$\begin{aligned}\lambda_l(\boldsymbol{\theta_2}) &= \frac{-(k_2+k_{-2}+k_d)+\sqrt{\Delta(\boldsymbol{\theta_2})}}{2},\\ \lambda_s(\boldsymbol{\theta_2}) &= \frac{-(k_2+k_{-2}+k_d)-\sqrt{\Delta(\boldsymbol{\theta_2})}}{2},\end{aligned} \tag{5.4.28}$$

and

$$\Delta(\boldsymbol{\theta_2}) = (k_2+k_{-2}+k_d)^2 - 4k_dk_{-2} = (k_2+k_{-2}-k_d)^2 + 4k_dk_2. \tag{5.4.29}$$

As a result of (5.4.29), we note constraints that we will find useful later:

$$|k_2+k_{-2}-k_d| < \sqrt{\Delta(\boldsymbol{\theta_2})} < k_2+k_{-2}+k_d\,. \tag{5.4.30}$$

Let us consider (5.4.27) as form 1 of $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s)$ and note that we can obtain two other forms from it should we assume that pole-zero cancellation can occur. Using the denominator terms of (5.4.25) given in (5.4.26) we obtain

$$\hat{\boldsymbol{\phi}}(\boldsymbol{\theta}) \triangleq \left(\boldsymbol{\phi}_1^\top(\boldsymbol{\theta_1}),\, \varphi_0^{[2]}(\boldsymbol{\theta_2}),\, \varphi_1^{[2]}(\boldsymbol{\theta_2})\right)^\top, \tag{5.4.31}$$

which leads to $\hat{\mathcal{I}}(\mathcal{C},\hat{\boldsymbol{\phi}})_1 = \{\boldsymbol{\theta}\}$. We cannot improve this result by incorporating the numerator coefficients of (5.4.25) into (5.4.31) and redoing the test.

Remark 5.2. The condition contributed to the test by $\varphi_3^{[2]}(\boldsymbol{\theta})$ of (5.4.26), $y(t_1,\boldsymbol{\theta_1}) = y(t_1,\boldsymbol{\theta_1'})$, is subsumed under the essential test equation $y(t,\boldsymbol{\theta_1}) = y(t,\boldsymbol{\theta_1'})$, $\forall t \in \bar{\mathbb{R}}_+$. Hence $\varphi_3^{[2]}(\boldsymbol{\theta})$ does not provide any conditions on parameter values beyond those provided by $\boldsymbol{\phi}_1$.

Assuming that pole-zero cancellation of $(s-\lambda_s(\boldsymbol{\theta_2}))$ occurs in (5.4.27) gives form 2 of $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}$ as

$$\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s) = \frac{\varphi_1^{[2]}(\boldsymbol{\theta})}{s-\lambda_l(\boldsymbol{\theta_2})}, \quad s \in \mathrm{H}_{\lambda_l(\boldsymbol{\theta_2})}, \tag{5.4.32}$$

with λ_l as in (5.4.28) and $\varphi_1^{[2]}$ left unevaluated. Forming $\hat{\boldsymbol{\phi}}(\boldsymbol{\theta}) \triangleq \left(\boldsymbol{\phi}_1^\top(\boldsymbol{\theta_1}),\, \lambda_l(\boldsymbol{\theta_2})\right)^\top$, then $\hat{\mathcal{I}}(\mathcal{C},\hat{\boldsymbol{\phi}})_2 = \{\boldsymbol{\theta}\}$.

Assuming that pole-zero cancellation of $(s - \lambda_l(\boldsymbol{\theta_2}))$ occurs in (5.4.27) gives form 3 (the final alternative form) of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}$:

$$\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s) = \frac{\varphi_1^{[2]}(\boldsymbol{\theta})}{s - \lambda_s(\boldsymbol{\theta_2})}, \quad s \in \mathrm{H}_{\lambda_s(\boldsymbol{\theta_2})}, \tag{5.4.33}$$

with λ_s defined by (5.4.28) and $\varphi_1^{[2]}$ left unevaluated. Forming $\hat{\boldsymbol{\phi}}(\boldsymbol{\theta}) = \left(\boldsymbol{\phi}_1(\boldsymbol{\theta_1})^\top,\, \lambda_s(\boldsymbol{\theta_2})\right)^\top$, then $\hat{\mathcal{I}}(\mathcal{C}, \hat{\boldsymbol{\phi}})_3 = \{\boldsymbol{\theta}\}$.

Remark 5.3. When transferred into the time domain, (5.4.32) and (5.4.33) each describe exponential decay of response with $\varphi_1^{[2]}(\boldsymbol{\theta}) = y(t_1, \boldsymbol{\theta})$. As noted in Remark 5.2, the term does not add any new restrictions to a hypothetical identifiability test.

From $\hat{\mathcal{I}}(\mathcal{C}, \hat{\boldsymbol{\phi}})_i = \{\boldsymbol{\theta}\}$ for $i = 1, 2, 3$, we have $\mathcal{I}_{\max} \triangleq \{\boldsymbol{\theta}\}$ for all $\boldsymbol{\theta} \in \Theta$. Hence, the SCUII Algorithm allows us to infer that $\mathcal{C}$ is globally *a priori* identifiable.

Classification of the two-state conformational change model as globally *a priori* identifiable by the SCUII Algorithm in Whyte [94] was significant as this was only the second published classification of an optical biosensor interaction model structure.

Remark 5.4. The SCUII Algorithm obtained the set $\mathcal{I}_{\max}$ which contains fewer solutions than $\mathcal{I}(\mathcal{C}, \boldsymbol{\phi}_1)$. This shows that the algorithm is more useful than a consideration of only the structure representing the dynamics before the switching event. As a result, it may be useful in classifying other ULSS-1 structures representing optical biosensor experiments, as these typically do not introduce parameters after the switching time. In such cases, despite the SCUII Algorithm using conditions only from the denominator of $\mathcal{L}\{y^{[2]}\}(s)$, one may still obtain enough parameter information to obtain a satisfactory result.

Let us now consider the application of the SCReMI Algorithm to $\mathcal{C}$.

5.4.4 Application of the SCReMI Algorithm to $\mathcal{C}$

As we determined $\mathcal{I}(\mathcal{C}, \phi_1)$ in (5.4.24), we have reached Step 3 of the SCReMI Algorithm. In order to proceed, it is necessary to test $\mathcal{C}^{[2]}$ for generic minimality.

5.4.4.1 Arguments towards showing that $\mathcal{C}^{[2]}$ is generically minimal

We presented approaches to inferring generic minimality of the LTI structure representing the dynamics of the original ULSS-1 structure after the switching event in Section 4.5. We employ them in this section in efforts to establish whether or not $\mathcal{C}^{[2]}$ is generically minimal. This problem is a non-trivial test of the methods proposed.

We begin with application of the approach of Proposition 4.3. As this uses a simplified form of the cancellation conditions introduced in Proposition 4.2, its application is less algebraically complex. While the approach is not successful, it has the useful result of eliminating one case of possible pole-zero cancellation in $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}$ from further consideration.

In seeking a definitive solution to the minimality problem we turn to the approach of Proposition 4.2. Prior to embarking on this, we make a graphical comparison of the state trajectories of $\mathcal{C}^{[1]}$ over time for the cases where a cancellation condition does and does not hold. The difference between the trajectories for randomly chosen parameter values indicates that the cancellation condition cannot hold everywhere in the parameter space. From here, we apply a series of approaches that enable us to impose successively stronger restrictions on the situations under which a cancellation condition can hold.

First we show that the condition cannot hold for all time in general. We then show that there is at most one state which is a feasible solution to the cancellation condition. A conjecture that there is not any feasible solution to the cancellation condition is supported by numerical simulations. Encouraged by this, our recent advances in the use of Maple$^{\text{TM}}$ on lengthy symbolic expressions allow us to finally prove that the cancellation condition

holds only for sets of measure zero of the parameter space. Hence, $\mathcal{C}^{[2]}$ is generically minimal. This judgement allows us to proceed to classify $\mathcal{C}$ using the SCReMI Algorithm.

We preserve here the approaches we used in getting to this point for two reasons. First, the process shown may provide a template to guide the analysis of other ULSS-1 structures. Second, through incorporation of other results or parameter restrictions (such as that noted in Remark 5.1), it may be possible to modify approaches we present such that they can reach a conclusion more simply or efficiently.

5.4.4.1.1 Examination of the simplified cancellation conditions Recall zeros of the denominator of (5.4.25), $\lambda_l(\boldsymbol{\theta_2})$ and $\lambda_s(\boldsymbol{\theta_2})$, given in (5.4.28).

Figure 5.4.1 shows a compartmental diagram of the two-state conformational change model used to represent the association phase of an experiment. A compartmental system associated with a diagram of the type shown is termed a catenary compartmental system (see, for example, Godfrey [29]). Figure 5.4.1 is effectively a diagram of systems in $\mathcal{C}^{[1]}$, and deleting the link directed from the B compartment to the (AB) compartment gives a compartmental diagram for systems in $\mathcal{C}^{[2]}$. As a result, systems in $\mathcal{C}^{[2]}$ are also catenary. Following Godfrey [29, Page 32], the eigenvalues of a catenary system are real. This requires $\Delta(\boldsymbol{\theta_2}) \geq 0$ in (5.4.28). This expectation is consistent with published response curves for optical biosensor experiments as these do not show oscillatory behaviour.

Replacing s in the numerator of (5.4.25) by each of λ_l and λ_s defined by (5.4.28) in turn leads to two systems of cancellation conditions as outlined in (4.5.18). Our application allows us to impose a further restriction on the feasible states.

Remark 5.5. Analyte-ligand complex forms during the association phase of a kinetic experiment performed under usual conditions. Hence, it is unphysical to have an initial state having components $\xi_2 = \xi_3 = 0$. This trivial case is of no practical interest.

Recall the template for cancellation conditions outlined in Proposition 4.3. Following

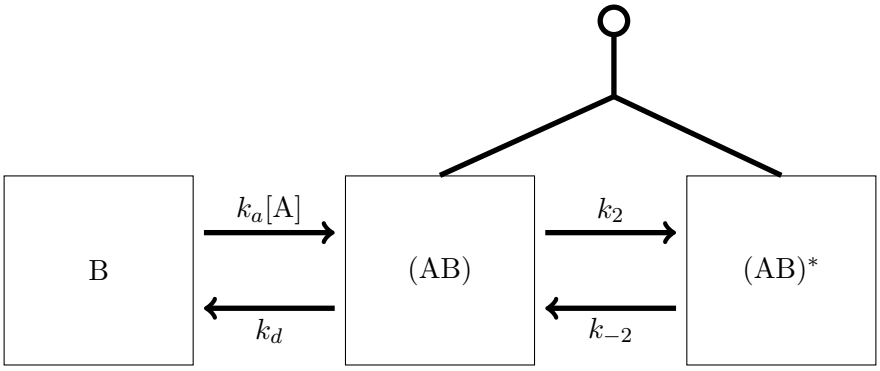


Figure 5.4.1: A compartmental diagram representing the association phase of an experiment under the two-state conformational change interaction model, as described in (5.4.18). The lines from the (AB) and (AB)* compartments show that these contribute to the single response function.

this, appropriate conditions for $\mathcal{C}^{[2]}$, a collection of closed compartmental systems obtained from (5.4.18) subject to Remark 5.5, are:

For $k = l, s$, determine solutions to

$$\begin{aligned} &\text{cancellation:} && \mathbf{C_2}(\boldsymbol{\theta}_2)\text{adj}(\lambda_k(\boldsymbol{\theta}_2)\mathbf{I}_3 - \mathbf{A_2}(\boldsymbol{\theta}_2))\boldsymbol{\xi} = 0, \\ &\text{subject to conditions of} \\ &\text{feasibility:} && \boldsymbol{\theta}_2 \in \mathbb{R}^3_+, \quad \xi_1 \geq 0, \quad \xi_2, \xi_3 > 0, \\ &\text{and physicality:} && \xi_1 + \xi_2 + \xi_3 = \beta_1. \end{aligned} \tag{5.4.34}$$

We present the details of an initial consideration of cancellation conditions in Section 5 of Appendix E. The condition after (5.4.34) for λ_l (shown in (5.4.28)) is

$$c_2\xi_2 + c_3\xi_3 = 0, \tag{5.4.35}$$

where

$$c_2 = (k_2 + k_{-2} - k_d) + \sqrt{\Delta(\boldsymbol{\theta_2})}, \quad c_3 = (k_2 + k_{-2} + k_d) + \sqrt{\Delta(\boldsymbol{\theta_2})}. \tag{5.4.36}$$

Recall from Remark 5.5 that $\xi_2, \xi_3 > 0$ and note that c_3 in (5.4.36) is positive. Let us consider the sign of c_2. Defining

$$Y = k_2 + k_{-2} - k_d \quad \text{allows} \quad c_2 = \sqrt{Y^2 + 4k_2k_d} + Y.$$

Using the constraint of (5.4.30),

$$c_2 = \sqrt{Y^2 + 4k_2k_d} + Y > |Y| + Y = \begin{cases} 0, & Y \leq 0, \\ 2Y, & Y > 0, \end{cases} \tag{5.4.37}$$

which shows that c_2 is positive.

However, as $\xi_2, \xi_3 > 0$ and $c_3 > 0$, c_2 must be negative in order to satisfy (5.4.35). Given this contradiction, there is no feasible (positive) combination of parameters and ξ_2 and ξ_3 that satisfy (5.4.35).

This shows that cancellation of $(s-\lambda_l)$ does not occur in the rational function (5.4.25) for feasible states or values of the parameters of practical interest.

We now turn our attention to the condition of (5.4.34) associated with λ_s,

$$d_2\xi_2 + d_3\xi_3 = 0, \tag{5.4.38}$$

where

$$d_2 = \frac{1}{2}\left(k_2 + k_{-2} - k_d - \sqrt{\Delta(\boldsymbol{\theta_2})}\right) \quad \text{and} \quad d_3 = \frac{1}{2}\left(k_2 + k_{-2} + k_d - \sqrt{\Delta(\boldsymbol{\theta_2})}\right).$$

Recall from Remark 5.5 that $\xi_2, \xi_3 > 0$ on physical grounds. Note that d_3 in (5.4.38) is $-\lambda_l > 0$. In order for (5.4.38) to have feasible solutions, d_2 must be negative. Using an argument similar to that of (5.4.37), again using $Y = k_2 + k_{-2} - k_d$ gives

$$d_2 = Y - \sqrt{Y^2 + 4k_2k_d} < Y - |Y| = \begin{cases} 2Y, & Y < 0, \\ 0, & Y \geq 0. \end{cases} \tag{5.4.39}$$

Hence, d_2 in (5.4.38) is always negative. As cancellation condition (5.4.38) has feasible solutions, we cannot dismiss cancellation of $(s - \lambda_s)$ in (5.4.26).

This result highlights a limitation of Proposition 4.3. In essence, the process used sacrifices detail in favour of simplicity by only placing non-negativity constraints on ξ_2 and ξ_3. However, the state vector is dependent on the parameter values. As such, it is not free to take any value in the plane of feasible states defined by the conditions of (5.4.34). Hence, determining meaningful solutions of the cancellation condition (5.4.34)

for λ_s may require the development of more realistic feasibility constraints for the states. Should adopting such conditions allow us to show that the cancellation condition (5.4.34) does not have physically realistic solutions, we could disregard cancellation of $(s - \lambda_s)$ in $\mathcal{L}\{y^{[2]}\}$. Given the ability of Proposition 4.3 to readily dismiss the cancellation condition associated with $(s - \lambda_l)$, more realistic (yet easy to derive) conditions may enhance the utility of the method described there for other problems. In future work we will consider the development of suitable conditions.

Moving from naïve state constraints to the most accurate ones directs us towards the use of the cancellation conditions of Proposition 4.2 rather than those associated with a variant of Proposition 4.3. We will proceed to consider the condition of Proposition 4.2 associated with λ_s. We benefit from not needing to consider the condition associated with λ_l due to the result above, which simplifies the problem. It also suggests that Proposition 4.3 can play a role as a first and simplest step in a multi-step approach to the consideration of cancellation conditions.

5.4.4.1.2 A graphical exploration of the remaining cancellation condition Let us consider the feasibility of a linear relationship between ξ_2 and ξ_3 as given by (5.4.38). As the state $\mathbf{x}^{[\mathbf{1}]}(t_1, \boldsymbol{\theta}_\mathbf{1})$ determines the $\boldsymbol{\xi}$ inherited by $\mathcal{C}^{[2]}(\boldsymbol{\theta})$ for a particular duration t_1 of the association phase of an experiment, let us consider the problem with reference to the behaviour of $\mathbf{x}^{[\mathbf{1}]}$.

Consider the implication of a relationship between state variables $x_2^{[1]}$ and $x_3^{[1]}$, such as $x_3^{[1]} = cx_2^{[1]}$ for some constant c, holding for some interval of time. This would suggest an uncoupling of the processes of interconversion of chemical species described by $\mathcal{C}^{[1]}$. As the chemical system is uncontrolled within a particular phase of an experiment, without some mechanism for this change of dynamics, the linear relationship seems unlikely. However, it seems more possible that (5.4.41) could hold for some isolated values of $x_2^{[1]}$ and $x_3^{[1]}$. If the condition (5.4.38) only holds for such isolated cases, it would be reasonable to suggest that in general (5.4.41) does not hold, and hence that cancellation of $(s - \lambda_s)$ does not

occur in general in (5.4.26). The following arguments draw on the first consideration of this problem, given in the presentation of Whyte [96].

Let us illustrate a case where the linear relationship between $x_2^{[1]}$ and $x_3^{[1]}$ given by (5.4.38) does not hold on some time interval. Consider the state trajectories of the system in $\mathcal{C}^{[1]}$ having parameter vector $\tilde{\boldsymbol{\theta}}$ of particular numeric values shown in Figure 5.4.2. Let us represent the state as a function of time by

$$\mathbf{r}_1(t, \tilde{\boldsymbol{\theta}}) \triangleq \begin{pmatrix} x_1(t, \tilde{\boldsymbol{\theta}}) & x_2(t, \tilde{\boldsymbol{\theta}}) & x_3(t, \tilde{\boldsymbol{\theta}}) \end{pmatrix}^\top \in \mathbb{R}^3_+, \tag{5.4.40}$$

where the state vector is given by (5.4.18) for $t \in [0, t_1)$. The blue line shows the state trajectory defined by (5.4.40), which connects the initial and equilibrium states.

Consider an alternative form of the condition given by Equation (5.4.38):

$$\xi_3 = K(\boldsymbol{\theta})\xi_2, \tag{5.4.41}$$

where, recalling (5.4.28),

$$0 < K = \frac{-k_2 - k_{-2} + k_d + \sqrt{\Delta(\boldsymbol{\theta_2})}}{(k_2 + k_{-2} + k_d) - \sqrt{\Delta(\boldsymbol{\theta_2})}} < \frac{k_d}{-\lambda_l}, \tag{5.4.42}$$

and positivity of the numerator and K overall is assured as a result of (5.4.39). Now consider (5.4.40) such that the physicality condition (effectively a conservation of mass condition) of (5.4.34) and the cancellation condition (5.4.41) hold. Accordingly, the behaviour of the state vector over time is given by

$$\mathbf{r}_2(t, \tilde{\boldsymbol{\theta}}) \triangleq \begin{pmatrix} x_1(t, \tilde{\boldsymbol{\theta}}) & \frac{1}{1+K}\left(\beta_1 - x_1(t, \tilde{\boldsymbol{\theta}})\right) & \frac{K}{1+K}\left(\beta_1 - x_1(t, \tilde{\boldsymbol{\theta}})\right) \end{pmatrix}^\top \in \mathbb{R}^3_+. \tag{5.4.43}$$

The khaki line in Figure 5.4.2 shows the state trajectory for $\mathbf{r}_2(\cdot, \tilde{\boldsymbol{\theta}})$. The only point of intersection of $\mathbf{r}_1(\cdot, \tilde{\boldsymbol{\theta}})$ and $\mathbf{r}_2(\cdot, \tilde{\boldsymbol{\theta}})$ is at $\mathbf{x}^{[\mathbf{1}]}(0, \boldsymbol{\theta})$, which is not a feasible value for $\boldsymbol{\xi}$ by Remark 5.5. This shows that (5.4.38) is not satisfied — even for a single feasible duration of the association phase of an experiment — on the state trajectory resulting from a particular parameter vector.

This observation indicates that relation (5.4.38) does not hold for all parameter values. This, and the physical reasoning earlier, encourages us to conjecture that the can-

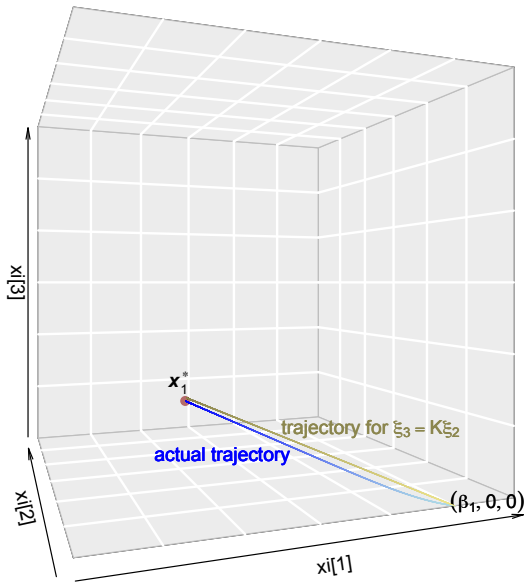


Figure 5.4.2: A comparison of state trajectories for the two-state conformational change model with $\boldsymbol{\theta_1} \triangleq (k_a, k_d, k_2, k_{-2}, \beta_1)$ taking the particular parameter values $\tilde{\boldsymbol{\theta}} = (2.5 \times 10^3, 3.5 \times 10^{-3}, 2 \times 10^{-2}, 3 \times 10^{-2}, 5)$ and $\alpha_1 = 1.5 \times 10^{-6}$, defining a particular system $\mathcal{C}^{[1]}(\tilde{\boldsymbol{\theta}})$. The initial state of the association phase, $\mathbf{x}^{[1]}(0, \tilde{\boldsymbol{\theta}}) = (\tilde{\beta}_1, 0, 0)^\top$, and equilibrium state $\mathbf{x}^{[1]^\star}(\tilde{\boldsymbol{\theta}})$ (the centre of the red circle) are shown. The blue trajectory shows the state evolution of $\mathcal{C}^{[1]}(\boldsymbol{\theta})$ as in (5.4.40). The khaki trajectory shows the state evolution when condition (5.4.41) is imposed on the states to give (5.4.43). The two trajectories only intersect at $\mathbf{x}^{[1]}(0)$.

cellation condition does not hold in general. To investigate this, next we will ascertain whether the condition can hold on a time interval.

5.4.4.1.3 Showing that the cancellation condition cannot hold on a time interval Let us consider the conditions under which (5.4.38) holds for a subinterval of $[0, +\infty)$, the time

domain to which t_1 belongs. This requires consideration of a set of initial conditions of $\mathcal{C}^{[2]}$ corresponding to an interval of durations of $\mathcal{C}^{[1]}$. This problem lends itself to the use of a symbolic algebra package. We provide the relevant calculations and a summary in Section 6 of Appendix E.

Let us consider the implications of (5.4.41) holding for some subinterval of the time set $[0, \infty)$ by reference to $\mathbf{x}^{[\mathbf{1}]}$. Recall the sum-of-exponentials form of the state function of a LTI system as in (3.6.28). Following this, we may express the states of $\mathcal{C}^{[1]}(\boldsymbol{\theta_1})$ as

$$\mathbf{x}^{[\mathbf{1}]}(t, \boldsymbol{\theta_1}) = e^{\mathbf{A_1}(\boldsymbol{\theta_1})t}\mathbf{x_0}(\boldsymbol{\theta_1}) = \sum_{i=1}^{3} \mathbf{v}_i e^{\lambda_i t}, \quad \text{where} \quad \mathbf{v}_i \triangleq \left(\mathbf{s}^{(i)'}\mathbf{x_0}\right)\mathbf{s}_i\,. \tag{5.4.44}$$

The largest eigenvalue of $\mathbf{A_1}$ is zero, denoted subsequently by λ_1. We use λ_2 and λ_3 to denote the other two eigenvalues, where $0 > \lambda_2 > \lambda_3$. (As these eigenvalues are distinct, this justifies the use of (5.4.44).) The eigenvector associated with λ_1, $\mathbf{s_1}$, (column 1 of $\mathbf{S}$) is far simpler in form than the other two eigenvectors. Further, the vector coefficient of $e^{\lambda_1 t}$ in (5.4.44) is $\mathbf{v_1} = (\mathbf{s}^{(1)'}\mathbf{x_0})\mathbf{s_1}$, which is $\mathbf{x}^{[\mathbf{1}]^*}(\boldsymbol{\theta_1})$, the equilibrium state of $\mathcal{C}^{[1]}(\boldsymbol{\theta_1})$. This is to be expected for the closed systems comprising $\mathcal{C}^{[1]}$, and is demonstrated by considering (5.4.44) in the limit as $t \to \infty$. Using this to combine (5.4.44) with (5.4.38) gives

$$a_1 e^0 + a_2 e^{\lambda_2 t} + a_3 e^{\lambda_3 t} = 0, \tag{5.4.45}$$

where

$$a_1 \triangleq d_2 x_2^{[1]*} + d_3 x_3^{[1]*}, \quad a_2 \triangleq d_2 v_{2_2} + d_3 v_{2_3} \quad \text{and} \quad a_3 \triangleq d_2 v_{3_2} + d_3 v_{3_3}.$$

We establish the linear independence of the set of functions $\{1, \exp(\lambda_2 t), \exp(\lambda_3 t)\}$ (λ_2 and λ_3 distinct and non-zero) by the standard method of computing the Wronskian of the three functions. Hence, if (5.4.45) is to have solutions on an interval, this requires the coefficients of all of the exponentials to be zero simultaneously.

The coefficients associated with $\exp(\lambda_2 t)$ and $\exp(\lambda_3 t)$ in (5.4.45) are quite complicated as a result of the complexity of the matrix of eigenvectors from which they are derived (see (5.4.44)). However, given the relative simplicity of the constant term a_1 in

(5.4.45), let us first consider when this term is zero. In order to motivate this choice, suppose $a_1 = 0$ for very few parameter values. Then, the cancellation condition is certainly not satisfied almost everywhere on a subinterval of $(0, \infty)$. We can make such a judgement without having to consider the other coefficients in (5.4.45).

To proceed with this investigation, substituting the expression for $\mathbf{x}^{[\mathbf{1}]^*}$ into $a_1 = 0$ and simplifying gives

$$-\frac{1}{2}\frac{k_a\alpha_1\beta_1}{k_a\alpha_1(k_2+k_{-2})+k_dk_{-2}}\left((k_2-k_{-2})k_d-(k_2+k_{-2})^2+(k_2+k_{-2})\sqrt{\Delta(\boldsymbol{\theta})}\right)=0. \tag{5.4.46}$$

Solving (5.4.46) for $\boldsymbol{\theta}$ gives the solution set

$$\Theta_{\text{null}} = \left\{\{\beta_1 = 0\}, \quad \{k_a = 0\}, \quad \{k_d = 0\}, \quad \{k_2 = 0\}, \quad \{k_{-2} = 0\}\right\}. \tag{5.4.47}$$

The set (5.4.47) is composed of five particular families of solution. Each is infeasible by the requirement that all parameters are strictly positive. As $a_1 \neq 0$, we have shown that the cancellation condition (5.4.38) does not hold on some subinterval of the time set $T = [0, \infty)$. We obtain the same result by treating ξ_2 and ξ_3 in (5.4.41) as functions of time defined for all $t \in [0, \infty)$ (with t representing the time of evolution of the association phase), applying the Laplace transform to (5.4.41), and determining the $\boldsymbol{\theta}$ for which the relationship is satisfied. We show this calculation under the heading "An alternative approach using the Laplace transform of $\mathbf{x}^{[\mathbf{1}]}$" in Section 6 of Appendix E.

We have shown that (5.4.41) does not hold for a continuum of states. Our next concern is whether the cancellation condition can have isolated feasible solutions.

5.4.4.1.4 The existence or otherwise of isolated solutions of the cancellation condition

The following proposition and its proof address the possibility that (5.4.41) holds for some isolated states ξ_2 and ξ_3.

Proposition 5.1. *Considering a particular $\boldsymbol{\theta_1} \in \Theta_1$, there is at most one feasible pair*

of states $(x_2^{[1]}(t,\boldsymbol{\theta_1}), x_3^{[1]}(t,\boldsymbol{\theta_1}))$ *which is a solution to the cancellation condition* (5.4.38) *(corresponding to* $k = s$ *in* (5.4.34)*).*

Proof. Recalling the revised form of the cancellation condition (5.4.38) given by (5.4.45), let us use the left-hand side to define a function for $t \in \bar{\mathbb{R}}_+$:

$$f(t,\boldsymbol{\theta}) \triangleq \sum_{i=1}^{3} a_i(\boldsymbol{\theta_2}) \exp\left(\lambda_i(\boldsymbol{\theta_1})t\right), \quad \lambda_3 < \lambda_2 < \lambda_1 = 0. \tag{5.4.48}$$

Aside from λ_1, the a_i and λ_i terms are complicated in form. (See Appendix E for these expressions. We show the numerator and denominator of a_2 in (E7.14) and (E7.6) respectively, and λ_2 in (E3.2.1.1).)

Now (5.4.38) is satisfied for t such that $f(t,\boldsymbol{\theta}) = 0$. This has a trivial solution at $t = 0$, which is infeasible (see Remark 5.5). We showed that it is not possible to have $f = 0$ on an interval of $\bar{\mathbb{R}}_+$ in Section 5.4.4.1.3.

If $f = 0$ for $t > 0$, then f must have a critical point. By simple differentiation, the condition for a critical point is

$$t \triangleq \frac{1}{\lambda_3 - \lambda_2} \ln\left(\frac{a_2\lambda_2}{-a_3\lambda_3}\right). \tag{5.4.49}$$

As $0 > \lambda_2 > \lambda_3$, the log term in (5.4.49) must be negative, as only positive values of t are relevant. This bounds the argument of the log term to lie in $(0,1)$. If the critical point corresponds to either a local maximum or a local minimum, then there is at most one $t \in (0,\infty)$ for which $f(t,\boldsymbol{\theta}) = 0$. If the critical point is a point of inflection, this does not give rise to a zero of f. Hence, there is at most one feasible solution to (5.4.38) as claimed. □

Let us consider the implications of Proposition 5.1 for when cancellation of $(s-\lambda_s)$ in $\mathcal{L}\{y^{[2]}\}$ may occur. Suppose we use $\mathcal{K}$ to denote the space of feasible values of $\boldsymbol{\theta_1}$, $\xi_2(\boldsymbol{\theta_1})$, and $\xi_3(\boldsymbol{\theta_1})$ satisfying the naïve constraints of (5.4.34). We note that for any feasible $\boldsymbol{\theta_1}$, the feasible $\xi_2(\boldsymbol{\theta_1})$ and $\xi_3(\boldsymbol{\theta_1})$ define a subset of $\mathcal{K}$ that is a bounded planar region in $\mathbb{R}^2$. The proof of Proposition 5.1 shows that for a given $\boldsymbol{\theta_1} \in \Theta_1$, the potential solution of

(5.4.34) for $\xi_2(\boldsymbol{\theta_1})$ and $\xi_3(\boldsymbol{\theta_1})$ defines at most a single point in this region. We will call any such point $(\hat{\xi_2}(\boldsymbol{\theta_1}), \hat{\xi_3}(\boldsymbol{\theta_1}))$. Let us use each $\boldsymbol{\theta_1} \in \Theta_1$ with a corresponding $(\hat{\xi_2}(\boldsymbol{\theta_1}), \hat{\xi_3}(\boldsymbol{\theta_1}))$ to define the set Щ, a proper subset of Ж. By definition, Щ is a subset of Ж of measure zero. Hence, we conclude that cancellation of $(s - \lambda_s)$ in $\mathcal{L}\{y^{[2]}\}$ can only occur for a subset of Ж of measure zero.

We can make a similar argument to establish when cancellation of $(s - \lambda_s)$ in $\mathcal{L}\{y^{[2]}\}$ occurs that, unlike the above argument, uses the actual nature of the set of states reached by a system in $\mathcal{C}^{[1]}$. For any feasible $\boldsymbol{\theta_1} \in \Theta_1$, the values achievable by $(x_2^{[1]}(t, \boldsymbol{\theta_1}), x_3^{[1]}(t, \boldsymbol{\theta_1}))$ for all $t \in \bar{\mathbb{R}}_+$ are restricted to a particular line segment in $\mathbb{R}^2_+$. We will use the feasible values of $\boldsymbol{\theta_1}$ and corresponding achievable $x_2^{[1]}(t, \boldsymbol{\theta_1})$ and $x_3^{[1]}(t, \boldsymbol{\theta_1})$ for $t \in \mathbb{R}_+$ to define Ӂ, a proper subset of Ж.

If Щ is a subset of Ӂ, then, by our definitions, Щ is a subset of Ӂ of measure zero. As Ӂ $\subset$ Ж, it is possible that for at least one $\boldsymbol{\theta_1} \in \Theta_1$, Ӂ does not include the $(\hat{\xi_2}(\boldsymbol{\theta_1}), \hat{\xi_3}(\boldsymbol{\theta_1}))$ for which (5.4.34) is satisfied. Hence, Ӂ $\cap$ Щ $\subset$ Щ, and the set of points in Ӂ for which cancellation of $(s - \lambda_s)$ in $\mathcal{L}\{y^{[2]}\}$ can occur is also a subset of Ӂ of measure zero.

From the discussion above we conclude that cancellation of $(s - \lambda_s)$ in $\mathcal{L}\{y^{[2]}\}$ can only occur for a subset of measure zero of the set composed of Θ_1 and the set of achievable initial states of $\mathcal{C}^{[2]}(\boldsymbol{\theta})$. We illustrate this result for a simplified case below.

For simplicity, let us consider systems from $\mathcal{C}^{[1]}$ with all parameters having a fixed value except for k_a. Similarly, let us only consider the values of ξ_2 and ξ_3, as these determine ξ_1. Under these conditions, we can display Ж in a three-dimensional graph as shown in the upper panel of Figure 5.4.3. As ξ_1 is not specified, we show the greatest range of values for ξ_2 and ξ_3 that could satisfy the constraints of (5.4.34). This is the interior of the shaded region. The figure also shows the intersection of Ж with a half-plane of constant k_a value.

The lower panel of Figure 5.4.3 shows this intersection in a two-dimensional plot.

The curved line shows an actual state trajectory traced out by ξ_2 and ξ_3 for a particular parameter vector. Question marks show the two possible types of unique solution for a point $(\hat{\xi}_2(k_a), \hat{\xi}_3(k_a))$ that can belong to the grey area, a subset of ЖК. (The dashed boundary is not included in ЖК.) A solution of the feasible type lies on the actual state trajectory, an infeasible solution does not. Recall from Proposition 5.1 that there may not be any solution for any particular k_a. Applying this logic over all cross-sections of ЖК caused by its intersection with a plane of feasible k_a value, we can see that the elements of ЩЦ form a subset of ЖК of measure zero.

We showed earlier that pole-zero cancellation of $(s - \lambda_l)$ in $\mathcal{L}\{y^{[2]}\}$ does not occur. This, taken with the above result on cancellation of $(s - \lambda_s)$, gives us reason to suspect that $\mathcal{C}^{[2]}$ is generically minimal. However, so far we have considered only a means of inferring solutions to (5.4.34). This is a simplified version of the problem of determining solutions to (5.4.38). We would prefer to use (5.4.38) to obtain a description of when $\mathcal{C}^{[2]}$ is minimal that is purely in terms of parameter values. Such an expression would allow us to adopt a more conventional and direct approach to classifying $\mathcal{C}^{[2]}$ as generically minimal or otherwise. However, we must overcome certain challenges in order to obtain such a result. We will consider these in the remainder of this section.

5.4.4.1.5 Further restricting the solution set of the cancellation condition: a conjecture and numerical support We may strengthen the result of Proposition 5.1 by showing that the condition on the argument of the log in (5.4.49) holds for at most a subset of the parameter space of measure zero. Approaching this problem using explicit expressions for a_2, λ_2, a_3 and λ_3 in (5.4.49) has exceeded the capability of Maple 16 to determine the solution set of a system of inequalities. Given this, we would like to ascertain if further efforts to address the problem are likely to be productive. To inform this decision, we advance a conjecture which we investigate through a computational approach.

Conjecture 5.1. For almost any $\boldsymbol{\theta_1} \in \Theta_1$, there is not any pair of states $(x_2^{[1]}(t, \boldsymbol{\theta_1}), x_3^{[1]}(t, \boldsymbol{\theta_1}))$ which is a feasible solution to the cancellation condition (5.4.38) (corresponding to $k = s$

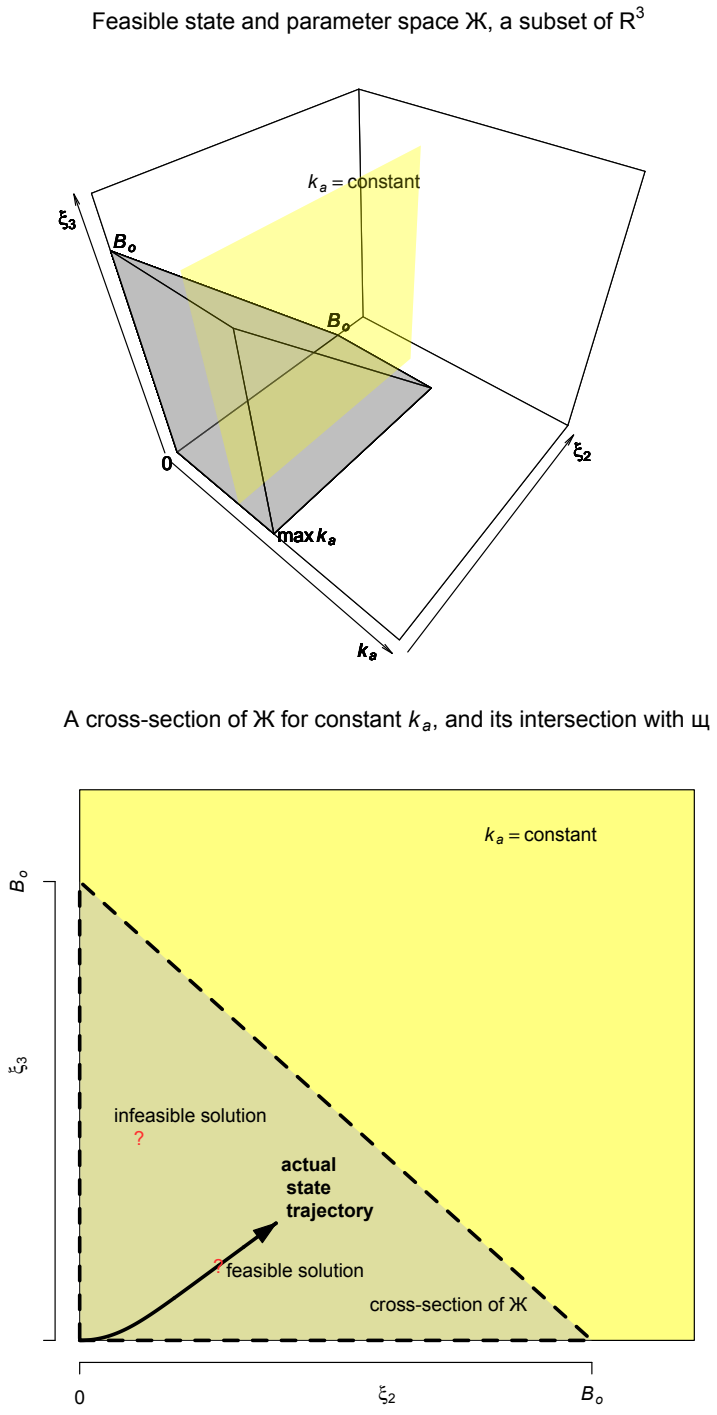


Figure 5.4.3: The space of reachable states of ξ_2 and ξ_3 and feasible values of k_a, Ж, for a one-parameter form of the two-state conformational change model structure $\mathcal{C}$. This aids our demonstration of an argument that pole-zero cancellation occurs in $\mathcal{L}\{y^{[2]}\}$ only for a subset of Ж of measure zero. The intersection of Ж with a plane of constant k_a value (top) and this intersection showing the actual state trajectory for the fixed parameter vector and possible solutions to the cancellation condition (bottom).

in (5.4.34)).

Support for the conjecture.

We devised a Maple program to undertake a computational investigation of solutions to the cancellation condition (5.4.38) for various parameter values. The program used a fixed analyte concentration α_1 and a uniform distribution with an appropriate range for each parameter to pseudo-randomly generate parameter values. Substitution of each set of these values into the cancellation condition resulted in an homogeneous equation in t. The method of solving any such equation restricted feasible solutions for t to the interval $[-1, 1000]$. We chose this time domain so as not to exclude solutions for t that were negative but close to 0. We set the upper value for t to 1000 seconds as experimental sensorgrams suggest this is often an adequate duration for an association phase.

Each execution of the programme consisted of attempts to solve 10^5 equations for t. All such root-finding attempts used the same number of digits in their calculations, determined by the value of the parameter d. We executed the programme for $d = 30$ and $d = 70$. To ensure reproducibility of results, we initialized the seed for the pseudo-random number generator to a particular documented value prior to the first programme execution. We present the Maple code and output in Appendix F.

Let t^* represent a solution returned by a root-finding process as described above. In the cases of each d value used, approximately 0.65% of runs were unable to return a value for t^*. This is possibly due to numerical matters, such as a failure of the search algorithm to converge to a solution within the maximum allowed number of iterations. Alternatively, for certain parameter combinations there may not be a solution $t^* \in [-1, 1000]$. The remaining runs all returned t^* that were very close to zero. Appendix F shows the spread of solutions with histograms of the $\log_{10}|t^*|$ values. These histograms show that $\log_{10}|t^*|$ values are rarely greater than $-d$, and hence that solutions returned are almost always closer to zero than $\pm 10^{-d}$ for $d = 30, 70$.

The close proximity of solutions to zero, with this closeness increasing as the number of digits used in the computations increases, and the lack of solutions away from zero, provide support for the conjecture. ◇

The support for Conjecture 5.1 encourages further effort to obtain an analytical argument that shows pole-zero cancellation in $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s)$ occurs only for a subset of the parameter space of measure zero.

5.4.4.1.6 A proof that the cancellation condition is rarely satisfied We were able to obtain some useful results in a recent study (Whyte [95]) through an enhancement of Maple's ability to collect terms in complicated expressions (see Appendix G). These permit us to make a stronger statement regarding structure $\mathcal{C}$.

Proposition 5.2. *For almost all* $\boldsymbol{\theta} \in \Theta$*, pole-zero cancellation cannot occur in* $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s)$ *as given by* (5.4.25)*.*

Proof. We present calculations required by this proof and numerical checks in Section 7 of Appendix E.

Recall that $\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s)$ given by (5.4.25) is equivalently expressed as (5.4.27). We showed earlier that cancellation of the factor $(s-\lambda_l)$ does not occur in (5.4.27). Hence, we must only consider the left-hand side of the cancellation condition for $(s-\lambda_s)$,

$$f(t,\boldsymbol{\theta}) \triangleq \sum_{i=1}^{3} a_i(\boldsymbol{\theta}) \exp(\lambda_i(\boldsymbol{\theta})t), \quad \lambda_3 < \lambda_2 < \lambda_1 = 0\,, \tag{5.4.48}$$

and show that it is not zero for $t > 0$ for almost all feasible parameter values.

Note that f is differentiable with respect to t for $t \in (0,\infty)$, and

$$\frac{df}{dt} = a_2\lambda_2 \exp(\lambda_2 t) + a_3\lambda_3 \exp(\lambda_3 t)\,,$$

with the one-sided derivative with respect to t as $t \to 0^+$ given by

$$\partial_+ f(0) = a_2\lambda_2 + a_3\lambda_3\,. \tag{5.4.50}$$

Let us first make some observations on (5.4.48). We have

$$f(0, \boldsymbol{\theta}) = a_1(\boldsymbol{\theta}) + a_2(\boldsymbol{\theta}) + a_3(\boldsymbol{\theta}) = 0\,, \tag{5.4.51}$$

as a consequence of states $x_2^{[1]}$ and $x_3^{[1]}$ being zero initially, (see Remark 5.5) and

$$\lim_{t \to +\infty} f(t, \boldsymbol{\theta}) = a_1(\boldsymbol{\theta})\,. \tag{5.4.52}$$

We proceed with the proof through arguments concerning the signs of a_1, a_2, and a_3.

Recall that the solutions for $a_1 = 0$ given in (5.4.47) show a_1 is not zero for any feasible parameter values. Inspection of a_1 given in (5.4.46) shows that it is the product of a clearly negative term and a second term currently of indeterminate sign. The first term has a denominator which is strictly positive, and hence this term is defined for all $\boldsymbol{\theta} \in \mathbb{R}^5_+$. The same judgement applies for the the second term as the term under the square root present is greater than zero in the feasible parameter space. As a consequence of this, the second term is differentiable with respect to $\boldsymbol{\theta}$, and hence continuous with respect to each parameter. As substitution of a particular feasible numerical value for $\boldsymbol{\theta}$ into the second term gives a positive value, we conclude that in the feasible parameter space this term is positive overall. As a result, $a_1 < 0$.

Given that $a_1 < 0$, let us consider possibilities for the behaviour of f which satisfy the conditions given by (5.4.51) and (5.4.52). There are two classes of possibilities; those where $\partial_+ f(0)$, is either positive or negative. These are shown schematically in Figure 5.4.4.

If $\partial_+ f(0) < 0$, then if f has a critical point at some $t_c > 0$ (cases IIa and IIb, dashed lines in Figure 5.4.4) it has the value $f_c < 0$. The critical point is a local minimum for $f_c < a_1$ and a point of inflection for $0 > f_c > a_1$. Regardless of whether f has a critical point or not (as for the dotted line, case IIc), as $f(t, \boldsymbol{\theta}) \to a_1 < 0$ as $t \to +\infty$, f does not meet the t-axis for any $t > 0$. Thus, in cases IIa,b,c, the cancellation condition is satisfied ($f(t, \boldsymbol{\theta}) = 0$) only at $t = 0$ which is an infeasible time by Remark 5.5.

If $\partial_+ f(0) > 0$, then f must have a local maximum at $t_c > 0$ in order to cross the t-axis at $t_\sharp$ as it tends to $a_1 < 0$ as $t \to +\infty$. This is shown by Case I, the solid line in

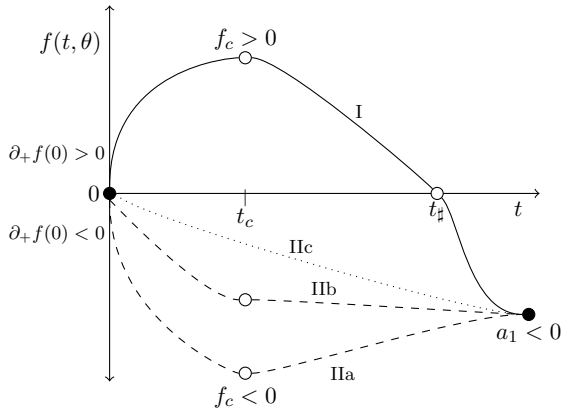


Figure 5.4.4: Feasible alternatives for f (as in (5.4.48)) given that $a_1 < 0$ for considering cases when cancellation condition (5.4.45) may hold. The function takes the values shown at the filled circles.

Figure 5.4.4, where a second t-intercept occurs for an arbitrary value of $\boldsymbol{\theta}$. In order to complete the proof, it is sufficient to show that Case I occurs only for some subset of the parameter space of measure zero. From this it follows that the only time at which the cancellation condition (5.4.38) is satisfied for almost all $\boldsymbol{\theta} \in \mathbb{R}^5_+$ is the infeasible $t = 0$.

Let us now consider the feasible $\boldsymbol{\theta}$ for which each possibility shown in Figure 5.4.4 can occur, with reference to a_2. As the expression for a_2 in terms of $\boldsymbol{\theta}$ is lengthy, we present it in Appendix E and merely refer to it here. In a similar manner as for a_1, we can consider a_2 through considering the factors of the expression as determined by Maple.

The denominator of a_2 is defined for all points in the feasible parameter space, that is, $\forall \boldsymbol{\theta} \in \mathbb{R}^5_+$. It is positive generally in the feasible parameter space except for where $k_{-2} = k_a \alpha_1$, in which case it is zero.

The numerator of a_2 is defined for all $\boldsymbol{\theta} \in \mathbb{R}^5_+$. It is only zero within the feasible parameter region when $k_{-2} = k_a \alpha_1$, as observed for the denominator. Hence, at $k_{-2} = k_a \alpha_1$, a_2 is of the indeterminate "0/0" form. Aside from this region of the parameter space, a_2 is defined by a negative numerator on a positive denominator, making $a_2 < 0$

when it is defined for some $\boldsymbol{\theta} \in \mathbb{R}^5_+$.

When $a_1, a_2 < 0$, then from (5.4.51)

$$a_3 = -(a_1 + a_2) > 0\,. \tag{5.4.53}$$

Recall the condition on the existence of a feasible critical point of f arising from (5.4.49)

$$0 < \frac{a_2\lambda_2}{-a_3\lambda_3} < 1\,. \tag{5.4.54}$$

As a consequence of (5.4.53) and $\lambda_3 < 0$, $-a_3\lambda_3 > 0$. Hence, rearranging (5.4.54) gives $a_2\lambda_2 < -a_3\lambda_3$, and thus

$$a_2\lambda_2 + a_3\lambda_3 < 0\,. \tag{5.4.55}$$

Equation (5.4.55) shows that when f has a critical point, it also has $\partial_+ f(0, \boldsymbol{\theta}) < 0$ by (5.4.50). Thus, Case I cannot occur if f has a critical point (that is, (5.4.54) holds), in which case Cases IIa or IIb occur. If f does not have a critical point, then only Case IIc occurs. Thus, for almost all $\boldsymbol{\theta} \in \mathbb{R}^5_+$ one of Cases IIa, IIb or IIc hold. Thus, pole-zero cancellation does not occur in $\mathcal{L}\{y^{[2]}\}$ for almost all $\boldsymbol{\theta} \in \mathbb{R}^5_+$. □

It is useful to summarise the insights obtained in this subsection. The cancellation condition for $(s - \lambda_l)$ does not hold anywhere in the parameter space. We showed that the equivalent cancellation condition for $(s - \lambda_s)$ does not hold for almost all feasible parameter values. Taken together, this shows that pole-zero cancellation in the unprocessed form of $\mathcal{L}\{y^{[2]}\}$ occurs only for a subset of the parameter space of measure zero. Hence $\mathcal{C}^{[2]}$ is generically minimal. We can now proceed to apply the SCReMI Algorithm to $\mathcal{C}$.

5.4.4.2 Classification of $\mathcal{C}$ using incomplete information from $\mathcal{C}^{[2]}$

Showing that $\mathcal{C}^{[2]}$ is generically minimal allows us to collect the denominator coefficients of $\mathcal{L}\{y^{[2]}\}$, as required by Step 3 of the SCReMI Algorithm. Recall that the algorithm requires us to combine parameter information obtainable from $\mathcal{C}^{[2]}$ with that from $\mathcal{C}^{[1]}$

to form $\grave{\boldsymbol{\phi}} \triangleq (\boldsymbol{\phi}_1(\boldsymbol{\theta}_1)^\top, \varphi_0^{[2]}(\boldsymbol{\theta}), \varphi_1^{[2]}(\boldsymbol{\theta}))^\top$. (We do not use the numerator coefficients of $\mathcal{L}\{y^{[2]}\}$, $\varphi_2^{[2]}(\boldsymbol{\theta})$ and $\varphi_3^{[2]}(\boldsymbol{\theta})$, due to their dependence on the initial conditions of $\mathcal{C}^{[2]}$.)

Proceeding to Step 4, we calculate the set

$$\mathcal{I}(\mathcal{C}, \grave{\boldsymbol{\phi}}) = \left\{ \boldsymbol{\theta}' \in \mathbb{R}_+^5 \,|k_a' = k_a,\ \beta_1' = \beta_1,\ k_d' = k_d,\ k_2' = k_2,\ k_{-2}' = k_{-2} \right\} \tag{5.4.56}$$

which shows that the SCReMI Algorithm has definitively classified $\mathcal{C}$ as globally *a priori* identifiable.

5.5 A review of results

The SCReMI Algorithm is able to classify the three-parameter form of the simple bimolecular interaction and the two-state conformational change model as globally *a priori* identifiable. In the case of the four-parameter simple bimolecular interaction model, the theory is not able to make a judgement on the model structure ($\mathcal{N}$). However, a supplementary and quite straightforward consideration of the nature of the structure shows that it is *a priori* unidentifiable. This result suggests a natural extension to the SCReMI Algorithm. When considering some ULSS-1 structure M, and creating LTI structures $M^{[1]}$ and $M^{[2]}$ from it, it is useful to inspect $\mathcal{I}(M, \grave{\boldsymbol{\phi}})$ to determine if there are inseparable parameter combinations which will ensure that M is *a priori* unidentifiable. If this is the case, we should reparameterise the original structure M.

Application of the SCUII Algorithm to the two-state conformational change model gives the same result as the SCReMI Algorithm with substantially less effort. However, as the SCUII Algorithm is not guaranteed to determine $\mathcal{I}(\mathcal{C}, \grave{\boldsymbol{\phi}})$, it is not a replacement for SCReMI Algorithm. Despite this, the result achieved for the test case demonstrates the usefulness of the SCUII Algorithm as either a quick preliminary approach to classifying a ULSS-1 structure, or possibly as a check on the result obtained by the SCReMI Algorithm. Further, the SCUII Algorithm readily lends itself to implementation in a symbolic algebra package, which will enable the automatic application of the algorithm to a ULSS-1

structure.

5.6 Prelude to the final chapter

Ultimately, use of the SCReMI Algorithm in classifying each of the three test cases gives definite results for structures representing specific flow-cell optical biosensor interaction models. However, we negotiated elements of the process in an *ad hoc* manner. In the next chapter we propose some additions to the theory that are intended to make the process of testing a ULSS-1 structure for global *a priori* identifiability more systematic. We also make some notes on directions for future research.

Chapter 6

New horizons

6.1 Overview

The approaches we proposed in Chapter 4 for testing a ULSS-1 structure for global *a priori* identifiability have proved adequate for classifying test cases drawn from the literature on flow-cell optical biosensor models. The works in progress outlined in this chapter suggest directions for development of the theory of Chapter 4. These works belong to two categories. In the first is a consideration of extensions to the SCUII Algorithm and SCReMI Algorithm to make them more efficient. The second category exploits a feature of mathematical models of flow-cell optical biosensor experiments which makes the testing methodology easier to apply in certain cases.

We also make brief comment on generalisations of the testing methodology to structures of linear switching systems of more than one switching event. The new directions, other uses of linear switching systems in biological modelling, and the range of flow-cell optical biosensor models yet to be formalised and tested for global *a priori* identifiability, serve as a stimulus for future studies.

6.2 The SCUII and SCReMI algorithms revisited

Recall from Section 4.4 that the SCUII Algorithm considers some number of alternative forms of the unprocessed form of $\mathcal{L}\{y^{[2]}\}$ in an attempt to make a judgement on a ULSS-1 structure. We expect this number to increase with the number of state variables. One means of improving the algorithm would be to reduce the number of forms of $\mathcal{L}\{y^{[2]}\}$ that we must consider. This would reduce the amount of effort required by an application of the test, and possibly result in us classifying a structure more easily.

Recall the application of the SCReMI Algorithm to the two-state conformational change model in Section 5.4.4. Consideration of the "cancellation conditions" in Section 5.4.4.1.1 showed that pole-zero cancellation of $(s - \lambda_l)$ in (5.4.25) is not feasible. Hence, a consideration of cancellation conditions prior to application of the SCUII Algorithm would exclude one form of $\mathcal{L}\{y^{[2]}\}$ from consideration. Further development of the study of cancellation conditions may augment the SCUII Algorithm such that it avoids considering infeasible forms of $\mathcal{L}\{y^{[2]}\}$.

We chose to use the package Maple in determining solutions of our cancellation conditions. We found that the Maple version originally available to us posed impediments to a satisfactory implementation of our planned approaches. One serious difficulty arose from Maple's method of constraining a constant to some feasible range via its "assume" facility. In our case, we required parameters and experimental variables to have positive values and each element of the initial condition vector to have a non-negative value. We expected that Maple's built-in solution methods applied to cancellation conditions having constrained variables would return only feasible solutions. Such a result would definitively determine the conditions under which pole-zero cancellation could occur in the Laplace transform of a response function as we consider in this thesis. However, we did not obtain this result.

Instead, we observed that from one Maple session to another we obtained different results for the same calculations. This caused us to abandon use of the assume facility

in our worksheets. Armando and Ballarin [2] subsequently diagnosed the type of inconsistency we observed as resulting from a problem with the implementation of the assume facility in certain Maple versions.

From our recent investigations, it appears that Maple continues to have difficulties with how the assume facility is used by other routines. For example, more recent Maple versions (Maple 14, 15, and 16.02a, the latest update available for version 16) may ignore conditions placed on variables by "assume" when solving equations. Hence, the solutions presented may relate to an unconstrained problem, rather than the desired constrained problem. As a result, we judged that we could not necessarily trust built-in methods of these Maple versions to correctly answer our questions relating to pole-zero cancellation.

We will revisit the solution of cancellation conditions using Maple through an updated version that has recently become available. Further, we will transpose the worksheets developed for this thesis to another symbolic algebra package to ascertain whether it is able to solve cancellation conditions subject to constraints. A positive finding may enable a more direct approach to the solution of cancellation conditions. This would contribute to both the efficiency of the SCReMI Algorithm and the SCUII Algorithm, as described earlier in this section.

Beyond the algebraic methods described above, we are considering some other methods. Consider a structure such as $\mathcal{C}^{[2]}$ in Section 5.4 for which each constituent system captures the behaviour exhibited by some system from $\mathcal{C}$ after the switching time. We are developing a process which aims to show that $\mathcal{C}^{[2]}$ is generically minimal through considering connectability of its compartments and physical reasoning. As results from this process are currently indicative rather than conclusive, they do not warrant inclusion in this thesis. We may gain further insights from the study of new test cases.

6.3 A special case: when the LTI structure representing ULSS-1 behaviour prior to the switching event reaches an equilibrium state

The SCReMI Algorithm attempts to classify a ULSS-1 structure M as globally *a priori* identifiable or otherwise by treating the switching time as a fixed but arbitrary positive value. This does not cause any problem for the analysis of the LTI structure $M^{[1]}$, representing the behaviour of M prior to the switching event. However, LTI structure $M^{[2]}$ representing M after the switching event has initial conditions that are unknown and not readily specified. It is this feature that causes much of the difficulty in testing a ULSS-1 structure for global *a priori* identifiability. If the initial conditions of $M^{[2]}$ could be specified simply as a constant vector, then this difficulty is removed. The remainder of this section will consider a special case arising in reversible chemical interactions — such as those studied in this thesis — which allows us to specify the initial conditions of $M^{[2]}$ in a straightforward manner. This leads to a simplified process for testing a ULSS-1 structure for global *a priori* identifiability, first described in Whyte [93].

Consider a flow-cell optical biosensor experiment where the interaction of analyte and ligand is described by the simple bimolecular model as formulated in Whyte [99]. We present a schematic of the idealised response for such an experiment in Figure 6.3.1.

Suppose the state of an experiment is defined by the concentrations of forms of complex and free ligand sites. Certain types of interaction systems reach a state of dynamic equilibrium (see Section 2.2.2) if the association phase of an experiment is of a sufficient duration. This feature is a characteristic of equilibrium experiments (recall Remark 2.1). We may expect a non-zero equilibrium state to occur as a consequence of the experimental conditions. We begin an experiment with a limited number of sites where analyte can bind to free immobilised ligand. This value is fixed if the system has a stable immobilisation level.[1] Assuming this is the case, and that analyte binds to ligand, the experimental condi-

[1]Some systems may not have this property, as we will see in Section 6.5.

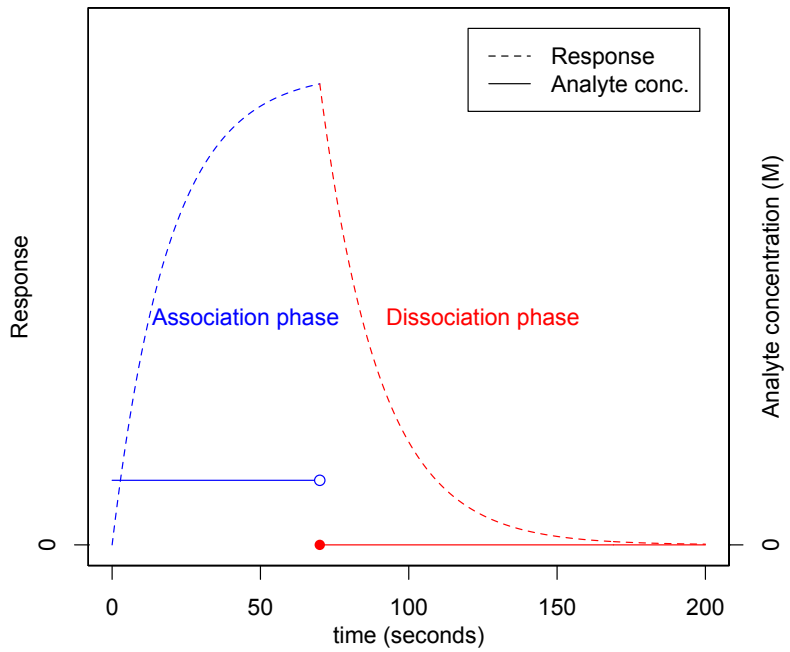


Figure 6.3.1: A schematic of the time course of analyte concentration and error-free response from a direct binding assay on a flow-cell optical biosensor. (Original version appeared in Whyte [94]. Permission to reuse IEEE content was approved for this purpose but any commercial reuse requires permission from IEEE, directly.)

tions determine the maximum (positive) amount of complex that can form. Once reached, the system remains at this state until there is a variation in experimental conditions, such as a change in the concentration of injected analyte.

In a sensorgram, such an equilibrium state is indicated by an equilibrium response, shown in Figure 6.3.2. This synthetic data was obtained from the system used to produce Figure 6.3.1 with the association phase given an increased duration.

The state and time at which an equilibrium state occurs depends on experimental variables and features of the interaction. These include the number of binding sites, injected analyte concentration, and rate constants, such as those relating to the association and dissociation of complex.

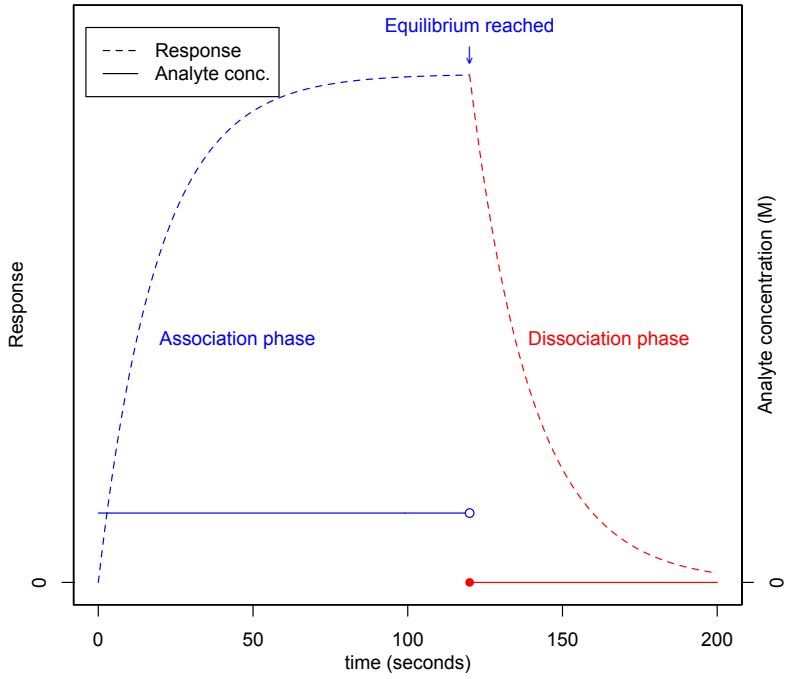


Figure 6.3.2: A schematic of the time course of analyte concentration and error-free response from a direct binding assay on a flow-cell optical biosensor experiment in which the association phase reaches an equilibrium state.

Recall our assumption that the state at the end of the association phase is the initial state of the dissociation phase. Consider the assumed ULSS-1 structure M, which we resolve into LTI structures $M^{[1]}$ and $M^{[2]}$ as described in Section 4.3.1. We obtain an algebraic expression for the equilibrium state attained in the association phase of an idealised experiment from $M^{[1]}(\boldsymbol{\theta}_1)$. Using this as the initial state of $M^{[2]}(\boldsymbol{\theta})$, we reduce the complexity of the problem of determining the canonical form of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$ to that of the equivalent problem for $M^{[1]}(\boldsymbol{\theta}_1)$, a standard LTI structure problem. Having determined the canonical form of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$, we can obtain the moment invariants present in the response of $M^{[2]}(\boldsymbol{\theta})$. This expectation suggests a new approach to testing a ULSS-1 structure for global *a priori* identifiability that eschews the complexity of the earlier approaches.

It is necessary to consider the circumstances under which a ULSS-1 from a structure representing a biomolecular interaction system may show an equilibrium state. Consider the ULSS-1 structure M (as in Definition 3.32) before the switching time t_1. Recall from Section 3.6.2.1 that the state predicted by some uncontrolled LTI system $M(\boldsymbol{\theta})$, $\mathbf{x}(\cdot, \boldsymbol{\theta})$, on the interval $[0, t_1)$ is described by a sum of exponentials having constant coefficients when the eigenvalues of its system matrix $\mathbf{A}$ are distinct. For simplicity, suppose that all parameters are introduced prior to the switching event. When the exponents of $\mathbf{x}(\cdot, \boldsymbol{\theta})$ on $t \in (0, t_1)$ are real and non-positive, $\mathbf{x}$ reaches an equilibrium state as t tends towards infinity. (This is the case here as the chemical systems considered are represented by catenary compartmental models with associated $\mathbf{A}$ a compartmental matrix following Definition 3.25.) However, it is not feasible to require this condition of M as the ULSS-1 systems it contains have a switching event at the finite time t_1, marking a change to the dynamics of the physical system in the dissociation phase. Taking t_1 to be infinite means that the dissociation phase does not commence, and hence that the accompanying data is not available to the test of the structure for global *a priori* identifiability.

We avoid this conceptual difficulty by assessing the properties of ULSS-1 structure M by consideration of uncontrolled LTI structures $M^{[1]}$ and $M^{[2]}$ derived from M. Recall that $M^{[1]}$ (Section 4.3.1) defined for a time set $T = \bar{\mathbb{R}}_+$ reproduces the behaviour of M on $[0, t_1)$, with state $\mathbf{x}^{[\mathbf{1}]}(t, \boldsymbol{\theta}) = \mathbf{x}(t, \boldsymbol{\theta})$ for $t \in [0, t_1)$. There is no conceptual problem in considering $\mathbf{x}^{[\mathbf{1}]}$ as time tends towards infinity to determine whether or not $\mathbf{x}^{[\mathbf{1}]}$ reaches an equilibrium state. As the matrix $\mathbf{A}_1(\boldsymbol{\theta})$ of $M^{[1]}(\boldsymbol{\theta})$ has non-positive eigenvalues, $M^{[1]}(\boldsymbol{\theta})$ has the equilibrium state

$$\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta}) = \lim_{t \to \infty} \mathbf{x}^{[\mathbf{1}]}(t, \boldsymbol{\theta}). \tag{6.3.1}$$

There are different ways to calculate $\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta})$. One way is to note that a fixed state occurs when $\dot{\mathbf{x}}^{[\mathbf{1}]}(\boldsymbol{\theta}) = \mathbf{0}$ in the state equation (4.3.8). Hence an equilibrium state is $\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta})$ that is a feasible solution of $\mathbf{A}_1(\boldsymbol{\theta})\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta}) = \mathbf{0}$.

In a flow-cell optical biosensor experiment, the equilibrium state of the association phase provides the initial state of the dissociation phase. Let us consider an idealised

dissociation phase that follows an association phase which has reached equilibrium. Accordingly, the initial state of $M^{[2]}(\boldsymbol{\theta})$ is $\mathbf{x}^{[\mathbf{2}]}(0,\boldsymbol{\theta}) \triangleq \mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta})$. Having obtained a simple expression for $\mathbf{x}^{[\mathbf{2}]}(0,\boldsymbol{\theta})$, we are able to obtain $\boldsymbol{\phi}_2(\boldsymbol{\theta})$ from $M^{[2]}(\boldsymbol{\theta})$, proceed to form $\boldsymbol{\phi}(\boldsymbol{\theta})$ as defined in Definition 4.1, and use this directly to test structure M for global *a priori* identifiability. Hence, this new approach overcomes the difficulty in obtaining $\boldsymbol{\phi}_2(\boldsymbol{\theta})$ faced by the SCReMI Algorithm, as observed in Section 5.4.2 when testing the two-state conformational change model for global *a priori* identifiability.

6.3.1 A new algorithm for testing a ULSS-1 structure for global *a priori* identifiability

We summarise the discussion of this section in the Equilibrium State Identifiability (ESI) algorithm for testing a ULSS-1 structure M for global *a priori* identifiability. The algorithm adapts the conditions required of M given in (4.2.4) of Definition 4.1 for the case where $\mathbf{x}^{[\mathbf{1}]^\star}$ is defined.

ESI Algorithm. Consider a ULSS-1 structure M composed of systems having real-valued states and outputs. Let M have feasible parameter set Θ. Suppose that all parameters are introduced in the representative system of M ($M(\boldsymbol{\theta})$ for unspecified $\boldsymbol{\theta} \in \Theta$) prior to the switching event.

1. Use M to define uncontrolled LTI structures $M^{[1]}$ and $M^{[2]}$ following Section 4.3.1. For $M^{[1]}(\boldsymbol{\theta})$, ascertain whether there exists an equilibrium state $\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta})$ (as defined by (6.3.1)) which is a feasible, non-trivial solution to $\mathbf{A_1}(\boldsymbol{\theta})\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta}) = \mathbf{0}$. If $\mathbf{x}^{[\mathbf{1}]^\star}$ exists, proceed to Step 2. If not, stop as this algorithm is not suitable.

2. Set $\mathbf{x}^{[\mathbf{2}]}(0,\boldsymbol{\theta}) \triangleq \mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta})$. Requiring that each system in $M^{[1]}$ and $M^{[2]}$ does not have a fixed state for all time places a condition on $M^{[1]}(\boldsymbol{\theta})$:

$$\dot{\mathbf{x}}^{[\mathbf{1}]}(0,\boldsymbol{\theta}) = \mathbf{A}_1(\boldsymbol{\theta})\mathbf{x}^{[\mathbf{1}]}(0,\boldsymbol{\theta}) \neq \mathbf{0}, \quad \forall \boldsymbol{\theta} \in \Theta, \tag{6.3.2}$$

   and another on $M^{[2]}(\boldsymbol{\theta})$:

$$\dot{\mathbf{x}}^{[\mathbf{2}]}(0,\boldsymbol{\theta}) = \mathbf{A}_2(\boldsymbol{\theta})\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta}) \neq \mathbf{0}, \qquad \forall \boldsymbol{\theta} \in \Theta. \tag{6.3.3}$$

Determine whether conditions (6.3.2) and (6.3.3) are satisfied. If so, proceed to Step 3. If not, stop.

3. Determine $\boldsymbol{\phi}_1(\boldsymbol{\theta})$ from the generically minimal form of $M^{[1]}$ (such as by collecting coefficients of the canonical form of $\mathcal{L}\{y^{[1]}(\cdot,\boldsymbol{\theta})\}$) and calculate $\mathcal{I}(M^{[1]},\boldsymbol{\phi})$ (see Definition 3.19). As $\mathcal{I}(M,\boldsymbol{\phi}) \subseteq \mathcal{I}(M^{[1]},\boldsymbol{\phi})$, if $|\mathcal{I}(M^{[1]},\boldsymbol{\phi}_1)| = 1$ then M is globally *a priori* identifiable and there is no need to proceed further. If $|\mathcal{I}(M^{[1]},\boldsymbol{\phi}_1)| > 1$ and the result of the test is not acceptable, response invariants of $M^{[2]}$ are required. Proceed to Step 4.

4. Obtain $\boldsymbol{\phi}_2(\boldsymbol{\theta})$ from the generically minimal form of $M^{[2]}$. Form the vector $\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq \langle \boldsymbol{\phi}_1(\boldsymbol{\theta}_1), \boldsymbol{\phi}_2(\boldsymbol{\theta}) \rangle$. Proceed to Step 5.

5. Determine $\mathcal{I}(M,\boldsymbol{\phi})$. Use this set to classify structure M by Definition 3.19.

Remark 6.1. The ESI Algorithm is suited to playing a role in the experimental design of flow-cell optical biosensor studies where a biomolecular interaction is known to reach an equilibrium state. However, for an interaction in which the rate constant of complex formation is small, an association phase might not reach equilibrium in a reasonable time. As such, the central assumption of the ESI Algorithm is not reasonable. This shows that the approaches of Chapter 4 — which do not require the state of the system to reach equilibrium prior to the switching event — are more general than the ESI Algorithm.

To illustrate the application of the ESI Algorithm, let us return to the three-parameter structure representing the simple bimolecular model given in Section 5.2.

6.3.2 Application of the ESI algorithm: an example

6.3.2.1 Extracting LTI structures $M^{[1]}$ and $M^{[2]}$ from the ULSS-1 structure M

To proceed with the application of ESI Algorithm to the three-parameter form of the simple bimolecular model given as a ULSS-1 structure in (5.2.2), let us denote the structure as M and use the process outlined in Section 4.3.1 to create LTI structures $M^{[1]}$ and $M^{[2]}$ from it.

6.3.2.2 The equilibrium state of LTI structure $M^{[1]}$

Systems in structure $M^{[1]}$ have two state variables; the structure's representative system $M^{[1]}(\boldsymbol{\theta_1})$ has state vector $\mathbf{x}^{[1]}(t,\boldsymbol{\theta_1}) = \left([B](t,\boldsymbol{\theta_1}),[AB](t,\boldsymbol{\theta_1})\right)^\top$ for parameter vector $\boldsymbol{\theta_1} = (\beta_1, k_a, k_d)^\top \in \mathbb{R}^3_+$. Let $\mathbf{x}^{[1]^\star}(\boldsymbol{\theta_1}) \triangleq (x_1^{[1]^\star}(\boldsymbol{\theta_1}), x_2^{[1]^\star}(\boldsymbol{\theta_1}))^\top$ denote an equilibrium state of $M^{[1]}(\boldsymbol{\theta_1})$. Solving $\mathbf{A_1}(\boldsymbol{\theta_1})\mathbf{x}^{[1]^\star}(\boldsymbol{\theta_1}) = \mathbf{0}$ for $\mathbf{x}^{[1]^\star}(\boldsymbol{\theta_1})$ gives solutions

$$\begin{bmatrix} x_1^{[1]^\star}(\boldsymbol{\theta_1}) \\ x_2^{[1]^\star}(\boldsymbol{\theta_1}) \end{bmatrix} = \begin{bmatrix} \frac{k_d}{k_a\alpha_1} \\ 1 \end{bmatrix} r, \quad r \in \mathbb{R}. \tag{6.3.4}$$

We require only those solutions in (6.3.4) that are feasible. First, we restrict the set of solutions to those that are positive. As parameters k_a and k_d and injected analyte concentration α_1 are positive, positive solutions of (6.3.4) require $r > 0$. Any state in $M^{[1]}$, a structure of closed compartmental models, is subject to a conservation of mass condition. In this application, it is more convenient to consider this condition through concentration variables. The initial concentration of functional immobilised ligand B, β_1 in (5.2.2), determines the maximum amount of complex AB that can form. As a result of the reaction scheme (5.2.1), the concentrations of immobilised ligand and complex in the closed system $M^{[1]}(\boldsymbol{\theta_1})$ are related by

$$[B](t,\boldsymbol{\theta}_1) + [AB](t,\boldsymbol{\theta}_1) = \beta_1. \tag{6.3.5}$$

Imposing (6.3.5) on (6.3.4) gives

$$r\left(\frac{k_d}{k_a\alpha_1}+1\right)=\beta_1. \tag{6.3.6}$$

Solution of (6.3.6) for r gives $r = k_a\alpha_1\beta_1/(k_a\alpha_1 + k_d)$, and using this in (6.3.4) leads to

$$\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta_1}) = \begin{bmatrix} \frac{k_d\beta_1}{k_a\alpha_1+k_d} \\ \\ \frac{k_a\alpha_1\beta_1}{k_a\alpha_1+k_d} \end{bmatrix}. \tag{6.3.7}$$

6.3.2.3 Check on whether M satisfies the conditions of ESI Algorithm, Step 2

The condition on $M^{[1]}(\boldsymbol{\theta_1})$ given by (6.3.2) is

$$\mathbf{A}_1(\boldsymbol{\theta_1})\mathbf{x}^{[\mathbf{1}]}(0,\boldsymbol{\theta_1}) = \begin{bmatrix} -k_a\alpha_1 & k_d \\ k_a\alpha_1 & -k_d \end{bmatrix}\begin{bmatrix} \beta_1 \\ 0 \end{bmatrix} = k_a\alpha_1\beta_1\begin{bmatrix} -1 \\ 1 \end{bmatrix} \neq \mathbf{0}\ \forall \boldsymbol{\theta_1} \in \mathbb{R}^3_+. \tag{6.3.8}$$

The corresponding condition on $M^{[2]}(\boldsymbol{\theta})$ follows from (6.3.3):

$$\mathbf{A}_2(\boldsymbol{\theta_2})\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta_1}) = \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix}\begin{bmatrix} \frac{k_d\beta_1}{k_a\alpha_1+k_d} \\ \\ \frac{k_a\alpha_1\beta_1}{k_a\alpha_1+k_d} \end{bmatrix} = \frac{k_ak_d\alpha_1\beta_1}{k_a\alpha_1+k_d}\begin{bmatrix} 1 \\ \\ -1 \end{bmatrix} \neq \mathbf{0}\ \forall \boldsymbol{\theta} \in \mathbb{R}^3_+. \tag{6.3.9}$$

As conditions (6.3.8) and (6.3.9) are each satisfied for all feasible parameter values, we proceed to Step 3 of the ESI Algorithm.

6.3.2.4 Consideration of structure $M^{[\mathbf{1}]}$ obtained from M

Recall from Section 5.2.1 that the vector of non-identically zero moment invariants obtainable from the response of $M^{[1]}(\boldsymbol{\theta_1})$ is given by $\boldsymbol{\phi}_1(\boldsymbol{\theta_1}) \triangleq \left(\phi_1^{[1]}(\boldsymbol{\theta_1}), \phi_2^{[1]}(\boldsymbol{\theta_1})\right)^\top$. Recall also that the set $\mathcal{I}(M, \boldsymbol{\phi}_1)$ has uncountably infinitely-many elements. As a result, we can make no useful judgement on M using only the observations of a system in M before the switching event. Hence, the test of M for global *a priori* identifiability requires us to proceed to obtain invariants from $M^{[2]}$ as described in Step 4 of the (ESI Algorithm).

6.3.2.5 Consideration of LTI structure $M^{[2]}$ obtained from M

From (4.3.12) the unprocessed Laplace transform of response of $M^{[2]}(\boldsymbol{\theta})$ is

$$\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s) = \mathbf{C_2}(\boldsymbol{\theta_2})(s\mathbf{I} - \mathbf{A_2}(\boldsymbol{\theta_2}))^{-1}\mathbf{x}^{[\mathbf{2}]}(0,\boldsymbol{\theta_1})\,.$$

Equating $\mathbf{x}^{[\mathbf{1}]^\star}(\boldsymbol{\theta_1})$ as defined by (6.3.7) with $\mathbf{x}^{[\mathbf{2}]}(0,\boldsymbol{\theta})$, we readily obtain the Laplace transform of $y^{[2]}(\cdot,\boldsymbol{\theta})$ in the canonical form as

$$\mathcal{L}\{y^{[2]}(\cdot,\boldsymbol{\theta})\}(s) = \frac{\phi_1^{[2]}(\boldsymbol{\theta})}{s+\phi_0^{[2]}(\boldsymbol{\theta})}, \quad s \in \mathrm{H}_{-k_d}, \tag{6.3.10}$$

where

$$\phi_0^{[2]}(\boldsymbol{\theta}) = -k_d \quad \text{and} \quad \phi_1^{[2]}(\boldsymbol{\theta}) = \frac{k_a\alpha_1\beta_1}{k_a\alpha_1 + k_d}\,. \tag{6.3.11}$$

Hence, we form the vector of moment invariants $\boldsymbol{\phi}_2(\boldsymbol{\theta}) \triangleq (\phi_0^{[2]}(\boldsymbol{\theta}), \phi_1^{[2]}(\boldsymbol{\theta}))^\top$.

6.3.2.6 A classification of M

We construct the vector of invariants $\boldsymbol{\phi}(\boldsymbol{\theta})$ obtainable from $M(\boldsymbol{\theta})$ by using $\boldsymbol{\phi}_1(\boldsymbol{\theta_1})$ (Equation (5.4.22)) and $\boldsymbol{\phi}_2(\boldsymbol{\theta})$ (Equation (6.3.11)) to give

$$\boldsymbol{\phi}(\boldsymbol{\theta}) = \left(k_a\alpha_1 + k_d, \quad k_a\alpha_1\beta_1, \quad -k_d, \quad \tfrac{k_a\alpha_1\beta_1}{k_a\alpha_1+k_d}\right)^\top. \tag{6.3.12}$$

As $M(\boldsymbol{\theta})$ has the nominal vector of parameters $\boldsymbol{\theta} = (k_a, k_d, \beta_1)^\top$, in order to form the equations of an *a priori* identifiability test let us first define the vector of alternative parameters (as seen in Equation (3.5.20)) as $\boldsymbol{\theta'} = (k'_a, k'_d, \beta'_1)^\top$. We now have all of the necessary elements so that we may proceed to determine $\mathcal{I}(M, \boldsymbol{\phi})$.

The equations of the test are defined by the relations $\boldsymbol{\phi}(\boldsymbol{\theta}) = \boldsymbol{\phi}(\boldsymbol{\theta'})$. By considering individual relations in turn, we may readily determine $\mathcal{I}(M, \boldsymbol{\phi})$. The equation corresponding to the third element in (6.3.12) is $k_d = k'_d$. Using this in the condition associated with the first element of (6.3.12) gives $k_a\alpha_1 + k_d = k'_a\alpha_1 + k_d$, hence $k'_a = k_a$. Finally, the condition resulting from the second element of (6.3.12) gives $k_a\alpha_1\beta_1 = k_a\alpha_1\beta'_1$, and hence

$\beta_1 = \beta_1'$. As a result, $\mathcal{I}(M, \boldsymbol{\phi}) = \{\boldsymbol{\theta}\}$ for all $\boldsymbol{\theta} \in \bar{\mathbb{R}}^3_+$, and we judge M as globally *a priori* identifiable.

Recall that we were able to use the SCReMI Algorithm to classify the two-state conformational change model as globally *a priori* identifiable in Section 5.4.4. We obtained the same classification from both the ESI Algorithm above and the SCUII Algorithm (Section 5.4.3). In applying these last two algorithms, we achieved a classification without having to negotiate the complexity required by application of the SCReMI Algorithm in its current state.

Let us designate a flow-cell optical biosensor kinetic experiment in which the association phase is allowed sufficient time to reach equilibrium as an AEK (Association Equilibrium Kinetic) experiment. We designed the ESI Algorithm specifically for the analysis of a structure representing such an experiment. We note that we may also use the SCUII Algorithm to classify a structure used to model AEK experiments. However, consider the case when a simple expression for $\mathbf{x}^{[\mathbf{2}]}(0, \boldsymbol{\theta})$ allows us to write $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$ in its canonical form. In this case we expect that the ESI Algorithm will provide a more efficient means of classifying a structure than the SCUII Algorithm as by using the former method we do not need to consider alternative forms of $\mathcal{L}\{y^{[2]}(\cdot, \boldsymbol{\theta})\}(s)$.

It is not standard practice for experimentalists to perform AEK experiments. However, adoption of these will mean that we can readily employ the ESI Algorithm as a means of anticipating the usefulness of a planned series of experiments.

6.4 Generalisation to LSS structures of multiple switching events

The standard type of kinetic experiment we consider in this thesis has one association phase followed by one dissociation phase. As typically an experimentalist performs a series of these experiments, one has to either wait for response to return to zero or perform a regeneration step (recall Figure 2.4.3) before starting the next experiment.

Other types of experiments are quite different from standard kinetic experiments. For example, there are variants of equilibrium experiments (recall Remark 2.1) which consist of a sequence of association phases with a different concentration of analyte used for each phase (Myszka *et al.* [56]). There are also experiments consisting of multiple cycles of association and dissociation phases in kinetic experiments where the surface is not regenerated between each cycle. A schematic of the analyte concentration over time for such an experiment is given in Figure 6.4.3. A schematic of response from a direct binding assay (see Section 2.4.2) with such a programmed time course of analyte concentration is given in Figure 6.4.4. By not requiring zero response prior to analyte injection, one would expect that such an experiment obtains data for parameter estimation more quickly than a series of the standard type of kinetic experiment.

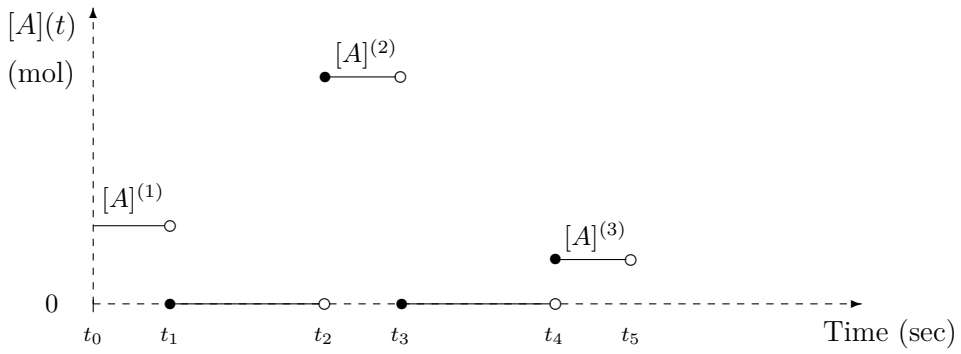


Figure 6.4.3: Time course of analyte concentration $[A]$ in an experiment having a series of cycles of association and dissociation phases.

Karlsson *et al.* [40] gave a particular form to the idea of an experiment composed of consecutive cycles of association and dissociation phases in the "kinetic titration" they proposed. The authors suggested that this type of experiment was appropriate when regeneration of the surface may inactivate the ligand surface, or when analyte and ligand were bound so strongly that an attempt to regenerate the surface would denature the ligand. In these experiments, the injected solution had a low analyte concentration in the first association phase, with this value increasing in each subsequent association phase. Results from fitting the simple biomolecular model to data presented in Karlsson *et al.* [40]

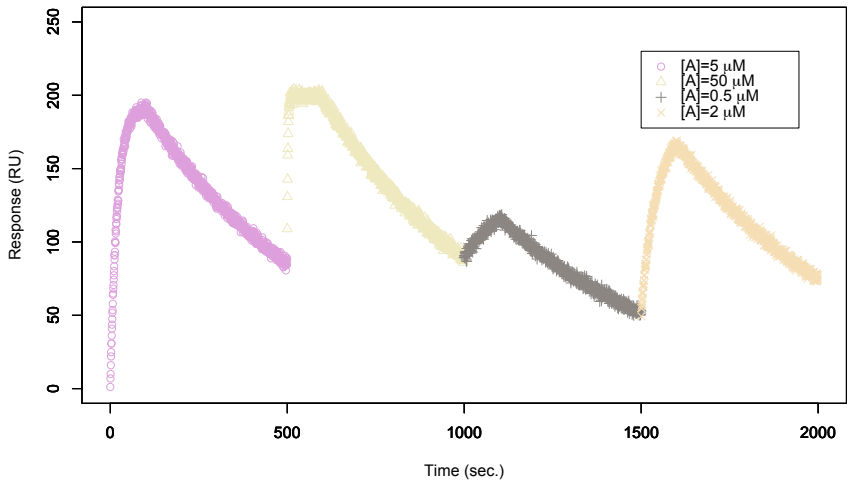


Figure 6.4.4: A schematic of the response of a direct binding assay on a flow-cell optical biosensor having successive cycles of association and dissociation phases.

showed that the estimates of rate constants made from data obtained from standard and kinetic titration experiments were comparable.

While Karlsson *et al.* [40] proposed kinetic titrations to avoid unsatisfactory features of the standard assay, different concerns have prompted other variants. Rich *et al.* [66] noted that optical biosensors are increasingly used in the initial phases of drug discovery programmes where one seeks to determine the affinity of candidate binding partners for a particular immobilised ligand. This has stimulated interest in how to increase the throughput of such programmes. Towards this end, in considering the features of the SensíQ biosensor (an SPR-based biosensor similar to models in the Biacore range) Rich *et al.* [66] considered "FastStep" injections. These require a series of injections of analyte at increasing concentrations, resulting in a series of association phases. The experiment concludes with one dissociation phase. A FastStep injection, like a kinetic titration, does not require surface regeneration.

Modelling a non-standard experiment such as those described above requires a struc-

ture of linear switching systems of more than one switching event. Accordingly, testing such a structure for global *a priori* identifiability requires a new approach. Extensions of the techniques we applied to ULSS-1 structures show promise for certain experimental situations.

Consider first an appropriate class of model structures for describing the interactions and response in non-standard experiments. Such a structure, say M, is composed of uncontrolled LSS of $J > 1$ switching events, now termed a ULSS-J structure, where the systems are defined for time set $T = [0, t_f]$. Suppose switching events occur at times $t_1, \ldots, t_J$, where $t_i < t_{i+1}$, $i = 1, \ldots, J-1$, where $t_1 > 0$ and $t_J < t_f$. By extension of the approach of Chapter 4, for $i = 1, \ldots, J+1$, let the LTI structure $M^{[i]}$ reproduce the time course of the state and output of M on the interval $t \in [t_{i-1}, t_i)$, where $t_0 = 0$ and $t_{J+1} = t_f$. For simplicity, suppose all parameters are introduced in the time interval $[t_0, t_1)$ and M has feasible parameter set Θ. For $\boldsymbol{\theta}$ an unspecified element of Θ, let $M^{[i]}(\boldsymbol{\theta})$ denote the representative system of $M^{[i]}$.

Testing a ULSS-J structure for global *a priori* identifiability in a classical manner requires us to generalise Definition 3.19. We achieve this by defining the vector of invariants $\boldsymbol{\phi}(\boldsymbol{\theta}) \triangleq (\boldsymbol{\phi}_1(\boldsymbol{\theta})^\top, \boldsymbol{\phi}_2(\boldsymbol{\theta})^\top, \ldots, \boldsymbol{\phi}_J(\boldsymbol{\theta})^\top)^\top$, where $\boldsymbol{\phi}_i(\boldsymbol{\theta})$ represents the moment invariants obtainable from $M^{[i]}(\boldsymbol{\theta})$. The descriptions of the Laplace transform of LTI system output given in Definition 4.2 and the associated moment invariants are sufficiently general so as to apply to the ULSS-J for $J \geq 1$.

Recall the discussion of Section 6.3 where we assumed that the association phase of a kinetic experiment reached equilibrium. We proposed the ESI Algorithm to test a ULSS-1 structure for global *a priori* identifiability under this condition. We showed that the ESI Algorithm was able to classify a structure as globally *a priori* identifiable more easily than the methods which did not assume that association reached an equilibrium state.

Following on from this insight, let us consider experiments of a kinetic titration or FastStep injection type. Suppose the experiments are designed such that each association phase reaches equilibrium. Consider the meaning of such experimental conditions for a

ULSS-J structure M representing the time evolution of a biomolecular interaction system. For $i = 1, \ldots, J+1$, the LTI $M^{[i]}(\boldsymbol{\theta})$ representing the idealisation of the experimental phase modelled by $M(\boldsymbol{\theta})$ on the interval $[t_{i-1}, t_i)$ reaches an equilibrium state as t tends to infinity. As a result, the initial conditions of each of $M^{[i]}(\boldsymbol{\theta})$ $i = 2, \ldots, J+1$ now have the desirable property of being represented by a constant vector. This removes the substantial barrier to obtaining the moment invariants that we encounter in the application of either the SCReMI Algorithm or SCUII Algorithm to a structure. Hence, an extension to the ESI Algorithm as applied to a ULSS-1 structure holds promise as a means of classifying a ULSS-J structure applicable to certain specific experimental conditions.

6.5 Concluding remarks

The "simple bimolecular interaction" and the "two-state conformational change interaction" are widely assumed mechanisms for biomolecular interactions occurring in direct binding assays performed on flow-cell optical biosensors. The developments which contributed to this thesis (Whyte [97–99]) have led to formalisation of the interaction models that has made model assumptions explicit.

This process has shown that each mechanism is appropriately represented by a structure of continuous-time, uncontrolled linear switching systems of one switching event. Testing such structures for the property of global *a priori* identifiability has received very little attention in the systems theory literature.

The algorithms we developed for this purpose have classified simplified structures representing the two aforementioned mechanisms as globally *a priori* identifiable. These are the first classifications of structures representing biomolecular interactions in flow-cell optical biosensor experiments. An alternative form of the simple bimolecular interaction having a greater number of parameters was classified as *a priori* unidentifiable. This result demonstrates the influence of the parameterisation on the properties of a structure. Further, this highlights the importance of clearly articulating descriptions of biosensor

experiments and interaction mechanisms as a necessary precursor to obtaining a model structure that is appropriate for use in estimating parameters from data.

In the domain of biomolecular interactions on flow-cell optical biosensors, there remain a number of interaction models that have not been formally specified. (See, for example, [34,41,42,51,58].) Some of these are also appropriately modelled by a LSS structure. The structures formalised in Whyte [97,99] provide a template to guide the complete specification of these. Having achieved this, we may also test these structures for global *a priori* identifiability using the approaches we presented in Chapter 4. We will consider this in future work.

In certain cases, we may expect that formalising model structures will proceed in a similar manner to the cases presented previously. One such example is that associated with an interaction system in which multiple analyte molecules of the same type can bind to an immobilised ligand molecule. As before, the system modelled has a constant total amount of immobilised ligand (that in free and bound forms). As a result, the response component due to the mass of ligand is constant. We can readily remove such a component from the response. This feature led to a simplification of both the original structure and its analysis in the examples we considered earlier.

However, modelling assays subject to less-than-ideal experimental conditions may entail new challenges. In particular, the "decaying surface model" (Joss *et al.* [38]) may be appropriate when immobilised ligand can dissociate from the sensor surface. In this case, the total immobilised ligand decreases over time. As such, the response component due to the mass of ligand is not constant. As a result, we cannot modify an initial structure to model processed data as readily as we could for structures considered earlier.

We are yet to formalise and test structures employed to describe a surface competition assay (SCA) or inhibition in solution (ISA) assay (see Section 2.4.2). To give some flavour of these, a simple form of SCA has heterogeneous analyte consisting of two species, say A_1 and A_2, with corresponding molecular masses M_{A_1} and M_{A_2}. Both analyte species are able to bind to the immobilised ligand B at rates influenced by rate constants that are

particular to each interaction. Suppose that only one analyte molecule can bind to each immobilised ligand molecule. Schematically we represent this interaction network by

$$\begin{array}{ccccc} & & \mathrm{A_2} & & \\ & & + & & \\ \mathrm{A_1} & + & \mathrm{B} & \underset{k_{d_1}}{\overset{k_{a_1}}{\rightleftharpoons}} & \mathrm{A_1B} \\ & & {\scriptstyle k_{d_2}}\uparrow\downarrow{\scriptstyle k_{a_2}} & & \\ & & \mathrm{A_2B} & & \end{array} \quad . \tag{6.5.13}$$

A simple form of ISA has a single species of inhibitor I able to bind analyte A, with the resulting complex (AI) unable to bind immobilised ligand B. We summarise this interaction system by the chemical network

$$\begin{array}{c} \mathrm{I} \\ + \\ \mathrm{A} + \mathrm{B} \rightleftharpoons (\mathrm{AB}) \\ \uparrow\downarrow \\ (\mathrm{AI}) \end{array} \quad . \tag{6.5.14}$$

Formulating suitably detailed structures for the interactions described above will provide additional test cases for our approaches to testing a ULSS-1 structure. Ideally, the ESI Algorithm (which imposes certain requirements on the idealised experimental response) or the less restrictive SCUII Algorithm will obtain definitive classifications of these test cases. Further, application of the SCReMI Algorithm to new structures may provide insights that guide its further development, resulting in a more directed means of classifying ULSS-1 structures.

Our original motivation for this thesis was a series of SCAs with interaction (6.5.13). One analyte species was of sufficiently low molecular weight that its binding to immobilised ligand was below the detection limit of the optical biosensor (see Section 2.3). Karlsson [41] presented a model structure describing such an interaction system and accompanying biosensor response. We assumed a similar type of structure for the interaction system, and conducted experiments to collect data for use in estimating its parameters.

We found that parameter estimation routines were unable to return useful results. The routines encountered regions of the parameter space where the objective function — the sum of squared differences between a data point and its predicted value — was virtually constant. Being unable to appreciably reduce the objective function in such a region, estimation routines terminated, issuing an error message. In attempting to diagnose the reason for this, close inspection of the structure revealed an inseparable combination of parameters. This is a symptom of an *a priori* unidentifiable model structure, which we could have anticipated prior to data collection should the field have employed such a practice. Such knowledge would have led to a reformulation of the assumed model structure. This in turn would have informed a more appropriate expectation of the parameter information obtainable from the planned experiments.

In this thesis we have considered only one area of modern molecular biology in which the unknown features of a biochemical process are modelled by a mathematical structure and observed indirectly on an apparatus. Another common example is the use of microarrays to indirectly observe the binding of RNA to DNA. Fluorescence data which includes a component due to formation of this complex is used with a model structure for binding rates (such as in Burden *et al.* [13]) to infer regulation of gene expression. A search of the literature indicates that researchers have not considered the testing such structures for global *a priori* identifiability.[2] We hope that the work presented in this thesis will increase awareness of the value of anticipating parameter estimation difficulties prior to data collection in such applications.

Contributions towards the design of experimental studies seem destined to grow in importance given the ongoing expansion of experimental hardware available, and concomitant growth in the volume of data produced by 'high-throughput' devices. To use the area that motivated this thesis as an example, there has been substantial growth in the variety of optical biosensors available for the study of biomolecular interactions. In 1998 and 1999

[2]For example, see Harrison *et al.* [33] for a recent review of research on the use of physico-chemical models in quantifying features of the binding of oligonucleotides on microarrays.

approximately 90% of publications relating to biosensors used Biacore instruments (Rich and Myszka [67]). However, in their 2010 review, Rich and Myszka [68, Table 1] presented 30 platforms.

Interest in a higher throughput means of monitoring binding interactions has led to a variant on the type of biosensor we considered in this thesis. Rather than having one sensor surface, these "Surface Plasmon Resonance Array Systems" (see, for example, Rich *et al.* [65]) have a 20×20 array of immobilised ligand samples. Each position in the array is monitored independently, allowing the study of a biomolecular interaction system under a range of conditions simultaneously rather than sequentially. Such an apparatus has the ability to hasten both the generation of a large volume of time series data and the consumption of resources. In such a setting, experimental design to obtain the most productive use of resources or an accurate understanding of the limitations of planned experiments has the potential to deliver an even greater efficiency dividend than it would have previously.

In their 2012 paper, Csercsik *et al.* [19] expressed a concern shared by various authors over a period of time:

> The importance of identifiability has been also stressed in the context of biological models [15-18]. However, many modeling and parameter estimation studies in computational biology still continue to ignore this key property.

In particular, researchers have paid little attention to testing structures of continuous-time linear switching systems for global *a priori* identifiability. This is a curious state of affairs given that their flexibility has lead to their use in modelling time-varying systems from a variety of applications (as we noted on Page 15).

Applications for switching systems also include the investigation of fundamental processes in biology. For example, Csercsik *et al.* [19] employed switching systems in modelling contributions to membrane current in voltage clamp experiments. This branch of electrophysiology does not commonly test the combination of a structure and set of experimental

conditions for global *a priori* identifiability. In order to simplify their problem, Csercsik *et al.* [19] considered the case where the voltage was assumed constant over the time of the experiment. Effectively this meant that the authors performed an *a priori* identifiability analysis for a time-invariant structure, rather than for a structure of switching systems.

Ideally researchers would be able to analyse a structure of the type that is most suited to their planned experiments, rather than a tractable approximation to it. Currently there is a need for a practical and systematic means of evaluating the suitability of switching system structures for their intended inverse problem. We will aim to extend our algorithms so that they can contribute to this matter.

The nature of a structure of linear switching systems makes it ill-suited to analysis by standard methods applied to linear time-invariant (LTI) structures or nonlinear structures. In this thesis we proposed testing a ULSS-1 structure for global *a priori* identifiability through various considerations of idealised LTI structures obtained from the ULSS-1 structure. The methods we proposed were adequate for classifying selected ULSS-1 structures. There is scope for further development of these methods.

A preliminary study inspired by the approaches we presented in this thesis suggests that further abstraction of the methods we proposed here, and an alternative formulation of global *a priori* identifiability for a ULSS-1 structure, will lead to a more elegant and systematic method of analysis. This approach should provide definitive results yet require us to negotiate less algebraic complexity than that inherent to application of the SCReMI Algorithm. Further, unlike the (ESI Algorithm), the revised approach will not require response to reach an equilibrium value. As a result, the approach in development shows promise for the analysis of structures of linear switching systems having multiple switching events. This will embolden the consideration of structures that have greater complexity than those we considered in this thesis. Favourable results will encourage us to deploy our methods in analysing structures from other disciplines.

Bibliography

[1] Making the most of the opportunities. *BIAjournal*, 1:10–11, 1994.

[2] Alessandro Armando and Clemens Ballarin. A reconstruction and extension of Maple's assume facility via constraint contextual rewriting. *Journal of Symbolic Computation*, 39:503–521, 2005.

[3] Stefania Audoly, Giuseppina Bellu, Leontina D'Angiò, Maria Pia Saccomani, and Claudio Cobelli. Global identifiability of nonlinear models of biological systems. *IEEE Transactions on Biomedical Engineering*, 48(1):55–65, 2001.

[4] David H. Bailey, Jonathan M. Borwein, and Alexander D. Kaiser. Automated simplification of large symbolic expressions. *Journal of Symbolic Computation*, 60:120–136, 2014.

[5] E. Batschelet, L. Brand, and A. Steiner. On the kinetics of lead in the human body. *Journal of Mathematical Biology*, 8(1):15–23, 1979.

[6] R. Bellman and K. J. Åström. On structural identifiability. *Mathematical Biosciences*, 7:329–339, 1970.

[7] Mones Berman. The formulation and testing of models. *Annals of the New York Academy of Sciences*, 108(1):182–194, 1964.

[8] BIACORE. *BIAapplications Handbook*. Pharmacia Biosensor AB, Uppsala, Sweden, 1994.

[9] El-Kébir Boukas. *Stochastic switching systems.* Control Engineering. Birkhäuser Boston Inc., Boston, MA, 2006. Analysis and design.

[10] George E. P. Box. Robustness in the strategy of scientific model building. In Robert L. Launer and Graham N. Wilkinson, editors, *Robustness in statistics : proceedings of a workshop sponsored by the Mathematics Division, Army Research Office, held at Army Research Office, Weiss Building, April 11-12, 1978*, pages 201–236, New York, 1979. Academic Press.

[11] George E. P. Box and Norman R. Draper. *Empirical model-building and response surfaces.* Wiley Series in Probability and Mathematical Statistics: Applied Probability and Statistics. John Wiley & Sons, Inc., New York, 1987.

[12] Yu. A. Brychkov, H.-J. Glaeske, A. P. Prudnikov, and Vũ Kim Tuấn. *Multidimensional integral transformations.* Gordon and Breach Science Publishers, Philadelphia, PA, 1992.

[13] Conrad J. Burden, Yvonne E. Pittelkow, and Susan R. Wilson. Statistical analysis of adsorption models for oligonucleotide microarrays. *Statistical Applications in Genetics and Molecular Biology*, 3(1), 2004.

[14] Peter E. Caines. *Linear Stochastic Systems.* John Wiley & Sons, Inc., 1988.

[15] R. Casey, H. De Jong, and J.-L. Gouzé. Piecewise-linear models of genetic regulatory networks: Equilibria and their stability. *Journal of Mathematical Biology*, 52(1):27–56, 2006.

[16] Raymond Chang. *Chemistry.* McGraw–Hill, New York, 4th edition, 1991.

[17] Harold Chestnut. Information requirements for systems understanding. *IEEE Transactions on Systems Science and Cybernetics*, SSC-6(1):3–12, January 1970.

[18] Louis Anthony Cox Jr. and William A. Huber. Symmetry, Identifiability, and Prediction Uncertainties in Multistage Clonal Expansion (MSCE) Models of Carcinogenesis. *Risk Analysis: An International Journal*, 27(6):1441–1453, 2007.

[19] D. Csercsik, K. M. Hangos, and G. Szederkényi. Identifiability analysis and parameter estimation of a single Hodgkin-Huxley type voltage dependent ion channel under voltage step measurement conditions. *Neurocomputing*, 77(1):178–188, 2012.

[20] B. Daya Reddy. *Introductory Functional Analysis : With Applications to Boundary Value Problems and Finite Elements*. Number 27 in Texts in Applied Mathematics. Springer, New York, 1998.

[21] Angela Dean, Daniel Voss, and Danel Draguljić. *Design and analysis of experiments*. Springer Texts in Statistics. Springer, Cham, Switzerland, 2nd edition, 2017.

[22] Lilianne Denis-Vidal and Ghislaine Joly-Blanchard. Equivalence and identifiability analysis of uncontrolled nonlinear dynamical systems. *Automatica*, 40(2):287–292, 2004.

[23] Jay L. Devore. *Probability and statistics for engineering and the sciences*. Belmont : Duxbury Press, 4th edition, 1995.

[24] Joseph DiStefano III. *Dynamic systems biology modeling and simulation*. Elsevier, Academic Press, Amsterdam, first edition, 2013.

[25] Gustav Doetsch. *Einführung in Theorie und Anwendung der Laplace-Transformation*. Birkhäuser Verlag, Basel, 1970. Ein Lehrbuch für Studierende der Mathematik, Physik und Ingenieurwissenschaft, Zweite, neubearbeitete und erweiterte Auflage, Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften. Mathematische Reihe, Band 24.

[26] J. Eisenfeld. Remarks on Bellman's structural identifiability. *Mathematical Biosciences*, 77:229–243, 1985.

[27] Jelel Ezzine and A. H. Haddad. Controllability and observability of hybrid systems. *International Journal of Control*, 49(6):2045–2055, 1989.

[28] GE Healthcare Life Sciences. Principle of Surface Plasmon resonance (SPR) used in Biacore™ systems, July 2013.

[29] Keith Godfrey. *Compartmental Models and Their Application.* Academic Press Inc., 1983.

[30] Keith R. Godfrey and Michael J. Chapman. Identifiability and indistinguishability of linear compartmental models. *Mathematics and Computers in Simulation*, 32(3):273–295, 1990.

[31] William H. Greene. *Econometric analysis.* Boston ; London : Pearson, 7th ed., international ed. edition, 2012.

[32] Ya Guo and Jinglu Tan. Identifiability and identification of switched linear biological models. *Biosystems*, 118:31–38, 2014.

[33] Andrew Harrison, Hans Binder, Arnaud Buhot, Conrad J. Burden, Enrico Carlon, Cynthia Gibas, Lara J. Gamble, Avraham Halperin, Jef Hooyberghs, David P. Kreil, Rastislav Levicky, Peter A. Noble, Albrecht Ott, B. Montgomery Pettitt, Diethard Tautz, and Alexander E. Pozhitkov. Physico-chemical foundations underpinning microarray and next-generation sequencing experiments. *Nucleic Acids Research*, 41(5):2779–2796, 2013.

[34] Anders Heding, Raj Gill, Yasushi Ogawa, Pierre De Meyts, and Ronald M. Shymko. Biosensor Measurement of the Binding of Insulin-like Growth Factors I and II and Their Analogues to the Insulin-like Growth Factor-binding Protein-3. *The Journal of Biological Chemistry*, 271(24):13948–13952, 1996.

[35] V. Hovelaque, C. Commault, and J. M. Dion. Zeros of structured linear systems. *Automatica*, 35(10):1683–1688, 1999.

[36] John A. Jacquez. The inverse problem for compartmental systems. *Mathematics and Computers in Simulation*, 24(452–459), 1982.

[37] John A. Jacquez and Peter Greif. Numerical parameter identifiability and estimability: Integrating identifiability, estimability and optimal sampling design. *Mathematical Biosciences*, 77(1-2):201–227, 1985.

[38] Lisa Joss, Thomas A. Morton, Michael L. Doyle, and David G. Myszka. Interpreting kinetic rate constants from optical biosensor data recorded on a decaying surface. *Analytical Biochemistry*, 261:203–210, 1998.

[39] R. E. Kalman. Mathematical description of linear dynamical systems. *SIAM Journal on Control and Optimization*, 1(2):152–192, 1963.

[40] R. Karlsson, P. S. Katsamba, H. Nordin, E. Pol, and D. G. Myszka. Analyzing a kinetic titration series using affinity biosensors. *Analytical Biochemistry*, 349(1):136–147, 2006.

[41] Robert Karlsson. Real-time competitive kinetic analysis of interactions between low-molecular-weight ligands in solution and surface-immobilized receptors. *Analytical Biochemistry*, 221:142–151, 1994.

[42] Robert Karlsson and Anders Fält. Experimental design for kinetic analysis of protein-protein interactions with surface plasmon resonance biosensors. *Journal of Immunological Methods*, 200:121–133, 1997.

[43] Robert Karlsson, Mari Kullman-Magnusson, Markku D. Hämäläinen, Annika Remaeus, Karl Andersson, Peter Borg, Erika Gyzander, and Johanna Deinum. Biosensor analysis of drug-target interactions: Direct and competitive binding assays for investigation of interactions between thrombin and thrombin inhibitors. *Analytical Biochemistry*, 278:1–13, 2000.

[44] Robert Karlsson, Anne Michaelsson, and Lars Mattsson. Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *Journal of Immunological Methods*, 145:229–240, 1991.

[45] Robert Karlsson, Håkan Roos, Lars Fägerstam, and Björn Persson. Kinetic and Concentration Analysis Using BIA Technology. *METHODS: A Companion to Methods in Enzymology*, 6:99–110, 1994.

[46] Dean C. Karnopp, Donald L. Margolis, and Ronald C. Rosenberg. *System Dynamics*. John Wiley & Sons, Inc., Hoboken, N. J., 5th edition, 2012.

[47] Ching-Tai Lin. Structural controllability. *IEEE Transactions on Automatic Control*, AC-19(3):201–208, 1974.

[48] Lennart Ljung and Torkel Glad. *Modeling of dynamic systems*. Prentice-Hall information and system sciences series. PTR Prentice Hall, Englewood Cliffs, N. J., 1994.

[49] S. Löfas and B. J. Johnsson. A novel hydrogel matrix on gold surfaces in surface plasmon resonance sensors for fast and efficient covalent immobilization of ligands. *Journal of the Chemical Society, Chemical Communications*, 121:1526–1528, 1990.

[50] Maplesoft, a division of Waterloo Maple Inc., Waterloo, Ontario. Maple 16.02a.

[51] Thomas A. Morton and David G. Myszka. Kinetic Analysis of Macromolecular Interactions Using Surface Plasmon Resonance Biosensors. *Methods in Enzymology*, 295:268–294, 1998.

[52] D. G. Myszka. Survey of the 1998 optical biosensor literature. *Journal of Molecular Recognition*, 12(6):390–408, Nov-Dec 1999.

[53] David G. Myszka. Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors. *Current Opinion in Biotechnology*, 8:50–57, 1997.

[54] David G. Myszka. Improving biosensor analysis. *Journal of Molecular Recognition*, 12:279–284, 1999.

[55] David G. Myszka, Xiaoyi He, Micah Dembo, Thomas A. Morton, and Byron Goldstein. Extending the Range of Rate Constants Available from BIACORE: Interpreting Mass Transport-Influenced Binding Data. *Biophysical Journal*, 75:583–594, 1998.

[56] David G. Myszka, Mathew D. Jonsen, and Barbara J. Graves. Equilibrium analysis of high affinity interactions using biacore. *Analytical Biochemistry*, 265:326–330, 1998.

[57] David G. Myszka and Thomas A. Morton. CLAMP: a biosensor kinetic data analysis program. *Trends in Biochemical Sciences*, 23:149–150, 1998.

[58] David G. Myszka, Thomas A. Morton, Michael L. Doyle, and Irwin M. Chaiken. Kinetic analysis of a protein antigen-antibody interaction limited by mass transport on an optical biosensor. *Biophysical Chemistry*, 64:127–137, 1997.

[59] Daniel J. O'Shannessy and Donald J. Winzor. Interpretation of Deviations from Pseudo-First-Order Kinetic Behaviour in the Characterization of Ligand Binding by Biosensor Technology. *Analytical Biochemistry*, 236:275–283, 1996.

[60] Mihaly Petreczky, Laurent Bako, and Jan H. van Schuppen. Identifiability of Discrete-Time Linear Switched Systems. In *HSSC 10: Proceedings of the 13th ACM International Conference on Hybrid Systems: Computation and Control*, pages 141–150. ACM SIGBED, Assoc Computing Machinery, 2010. Sweden, Apr 12-15, 2010.

[61] Phuoc Vo Tan, G. Millerioux, and J. Daafouz. Invertibility, flatness and identifiability of switched linear dynamical systems: an application to secure communications. In *47th IEEE Conference on Decision and Control*, pages 959–964, Piscataway, N. J., USA, 2008. IEEE. 9-11 Dec., Cancun, Mexico.

[62] Phuoc Vo Tan, G. Millerioux, and J. Daafouz. Left invertibility, flatness and identifiability of switched linear dynamical systems: a framework for cryptographic applications. *International Journal of Control*, 83:145–153, Jan 2010.

[63] Svatopluk Poljak. On the gap between the structural controllability of time-varying and time-invariant systems. *IEEE Transactions on Automatic Control*, 37(12):1961–1965, 1992.

[64] Atilla Raksanyi, Yves Lecourtier, Eric Walter, and Alain Venot. Identifiability and distinguishability testing via computer algebra. *Math. Biosci.*, 77(1-2):245–266, 1985.

[65] R. L. Rich, M. J. Cannon, J. Jenkins, P. Pandian, S. Sundaram, R. Magyar, J. Brockman, J. Lambert, and D. G. Myszka. Extracting kinetic rate constants from surface plasmon resonance array systems. *Analytical Biochemistry*, 373(1):112–120, 2008.

[66] R. L. Rich, J. G. Quinn, T. Morton, J. D. Stepp, and D. G. Myszka. Biosensor-based fragment screening using faststep injections. *Analytical Biochemistry*, 407(2):270–277, 2010.

[67] Rebecca L. Rich and David G. Myszka. Advances in surface plasmon resonance biosensor analysis. *Current Opinion in Biotechnology*, 11:54–61, 2000.

[68] Rebecca L. Rich and David G. Myszka. Grading the commercial optical biosensor literature — Class of 2008: 'The Mighty Binders'. *Journal of Molecular Recognition*, 23(1):1–64, Jan-Feb 2010.

[69] Rebecca L. Rich and David G. Myszka. Survey of the 2009 commercial optical biosensor literature. *Journal of Molecular Recognition*, 24(6):892–914, Nov-Dec 2011.

[70] R. T. Roper, M. P. Saccomani, and P. Vicini. Cellular signaling identifiability analysis: A case study. *Journal of Theoretical Biology*, 264(2):528–537, 2010.

[71] Keith R. Godfrey Sandor Vajda and Herschel Rabitz. Similarity transformation approach to identifiability analysis of nonlinear compartmental models. *Mathematical Biosciences*, 93:217–248, 1989.

[72] H. Schmidt, M. F. Madsen, S. Dano, and G. Cedersund. Complexity reduction of biochemical rate expressions. *Bioinformatics*, 24(6):848–854, 2008.

[73] G. A. F. Seber and C. J. Wild. *Nonlinear Regression.* John Wiley & Sons, 1989.

[74] E. Seneta. *Non-negative matrices and Markov chains.* Springer series in statistics. New York : Springer-Verlag, 2nd edition, 1981.

[75] Esa Stenberg, Björn Persson, Håkan Roos, and Csaba Urbaniczky. Quantitative Determination of Surface Concentration of Protein with Surface Plasmon Resonance Using Radiolabeled Proteins. *Journal of Colloid and Interface Science*, 143:513–526, 1991.

[76] David Sterratt, Bruce Graham, Andrew Gillies, and David Willshaw. *Principles of Computational Modelling in Neuroscience*. Cambridge University Press, 2011.

[77] Z. Sun and S. S. Ge. *Switched linear Systems: control and design*. Communication and Control Engineering. Springer-Verlag, London, 2005.

[78] C. Tomlin, G. J. Pappas, and S. Sastry. Conflict resolution for air traffic management: a study in multiagent hybrid systems. *IEEE Transactions on Automatic Control*, 43(4):509–521, Apr 1998.

[79] S. Vajda. Structural identifiability of linear, bilinear, polynomial and rational systems. In *Proceedings of the 9th IFAC World Congress, Budapest, Hungary*, page 107, 1984.

[80] S. Vajda and H. Rabitz. Identifiability and distinguishability of first-order reaction systems. *Journal of Physical Chemistry*, 92(3):701–707, 1988.

[81] J. M. van den Hof. Structural identifiability from input-output observations. Technical Report BS-9514, Centrum voor Wiskunde en Informatica, P. O. Box 94079, 1090 GB Amsterdam, The Netherlands, 1995.

[82] J. M. van den Hof. Structural identifiability of linear compartmental systems. *IEEE Transactions on Automatic Control*, AC-43(6):800–818, 1998.

[83] G. C. Vansteenkiste and J. A. Spriet. Structure/Parameter Identification by Simulation Experimentation. In M. G. Singh, editor, *System and Control Encyclopedia: theory, technology, applications*, volume 7, pages 4690–4692. Oxford; New York: Pergamon Press, 1987-1992.

[84] J. M. Varah. On fitting exponentials by nonlinear least squares. *Society for Industrial and Applied Mathematics Journal on Scientific and Statistical Computing*, 6(1):30–44, 1985.

[85] R. Vidal, A. Chiuso, and S. Soatto. Observability and identifiability of jump linear systems. In *Proceedings of the 41st IEEE Conference on Decision and Control*, volume 1-4, pages 3614–3619, N. Y., USA, Dec 10-13 2002. IEEE Control Syst Soc; Soc Ind & Appl Math; Inst Operat Res & Management Sci, IEEE. Las Vegas, N. V.

[86] Alejandro F Villaverde, Antonio Barreiro, and Antonis Papachristodoulou. Structural Identifiability of Dynamic Systems Biology Models. *PLoS Computational Biology*, 12(10):e1005153, 2016.

[87] Emilia Vynnycky and Richard White. *An introduction to infectious disease modelling*. Oxford University Press, 2010.

[88] Éric Walter and Luc Pronzato. *Identification of Parametric Models from Experimental Data*. Communication and Control Engineering. Springer, 1997.

[89] Gilbert G. Walter. Size identifiability of compartmental models. *Mathematical Biosciences*, 81:165–176, 1986.

[90] J. M. Whyte. On Deterministic Identifiability of Uncontrolled Linear Switching Systems. *WSEAS Transactions on Systems*, 6(5):1028–1036, May 2007.

[91] J. M. Whyte. A preliminary approach to deterministic identifiability of uncontrolled linear switching systems. In *3rd WSEAS International Conference on Mathematical Biology and Ecology (MABE'07)*, Proceedings of the WSEAS International Conferences, Gold Coast, Queensland, Australia, Jan 2007.

[92] J. M. Whyte. An improvement to the process of testing uncontrolled linear switching systems for *a priori* identifiability. Adelaide, South Australia, 3-6 December 2009. Presented at the Engineering Mathematics and Applications Conference (EMAC2009).

[93] J. M. Whyte. Simplifying the testing of models of flow-cell optical biosensor experiments for *a priori* identifiability. In *SIAM Conference on the Life Sciences (LS12) book of abstracts*, San Diego, U.S.A., 7-10 August 2012.

[94] J. M. Whyte. Inferring global *a priori* identifiability of optical biosensor experiment models. In Guo-Zheng Li, Xiaohua Hu, Sunghoon Kim, Habtom Ressom, Michael Hughes, Baoyan Liu, Geoff McLachlan, Michael Liebman, and Hongye Sun, editors, *IEEE International Conference on Bioinformatics and Biomedicine (IEEE BIBM 2013)*, pages 17–22, Shanghai, China, Dec 2013.

[95] J. M. Whyte. Global *a priori* identifiability of models for biomolecular interactions. Abstract accepted for the 8th Australia–New Zealand Mathematics Convention, Melbourne, 8-12 Dec 2014.

[96] J. M. Whyte. Minimality is a big deal for the analysis of biomolecular models. Osaka, Japan, 30 June 2014. Presented at the joint meeting of the Society for Mathematical Biology and the Japanese Society for Mathematical Biology.

[97] J. M. Whyte. Benefits of a transparent model relating SPR biosensor data to the "two-state conformational change" mechanism. Manuscript in progress, 2017.

[98] J. M. Whyte. The practical value of an explicit response model in quantitative uses of surface plasmon resonance biosensors. Manuscript in progress, 2017.

[99] J. M. Whyte. Resolving ambiguity in models of SPR biosensor data featuring the "simple bimolecular interaction". Manuscript in progress, 2017.

[100] J. M. Whyte, A. V. Metcalfe, M. A. Sugden, G. D. Abbott, and C. E. M. Pearce. *Advances In Statistics, Combinatorics and Related Areas*, chapter Estimation of Parameters in Pyrolysis Kinetics, pages 361–373. World Scientific, 2002.

[101] Takeo Yamada and David G. Luenberger. Generic Controllability Theorems for Descriptor Systems. *IEEE Transactions on Automatic Control*, AC-30(2):144–152, Feb 1985.

[102] James W. T. Yates. Structural Identifiability of Physiologically Based Pharmacokinetic Models. *Journal of Pharmacokinetics and Pharmacodynamics*, 33(4):421–439, 2006.

[103] R. Zazworsky and H. Knudsen. Controllability and observability of linear time-invariant compartmental models. *IEEE Transactions on Automatic Control*, 23(5):872–877, 1978.

[104] L.-Q. Zhang, J. C. Collins, and P. H. King. Indistinguishability and identifiability analysis of linear compartmental models. *Mathematical Biosciences*, 103(1):77–95, 1991.

[105] Qi Zheng. Computer algebra is indispensable in some problems of mathematical biology. *Mathematical Biosciences*, 151(2):219–225, 1998.

Appendices

Appendix A

Some fundamentals of chemistry

Moles and molar concentrations: useful measures of the amount of chemical species

In many reactions there are a large number of interacting species. As a result, a convenient measure of the number of atoms or molecules is moles (units mol) where one mole is 6.022045×10^{23} particles. This value is termed Avogadro's number. The molecular mass of some compound X, M_X, is defined as the mass of one mole of the substance, and has units of grams per mole (g mol^{-1}). The number of moles of X, n_X, present in a sample of mass m_X grams is given by

$$n_X = \frac{m_X}{M_X} \quad \text{mol.} \tag{A.0.1}$$

Often reactions occur between species present in some solution. When describing how much compound X is present in solution it is often convenient to consider the the number of moles of X present in a unit volume. This is the molar concentration of species X, denoted here by c_X. Given the number of moles of X, (n_X from (A.0.1)) in some volume V (standard units are dm^3) , the molar concentration of X is

$$c_X = \frac{n_X}{V} \quad \text{mol dm}^{-3} \, . \tag{A.0.2}$$

Appendix B

Maple Analysis of Structure 0

An example of testing a linear time-invariant structure for global *a priori* identifiability

We originally designed this worksheet for testing a structure of linear switching systems (LSSs) of one transition for global *a priori* identifiability.
A LSS in such a structure has a vector output $\boldsymbol{y}$ and a control $\mathbf{u}$ that is either a vector or a scalar. As a result, the Laplace transform of output for any component linear time-invariant (LTI) system defined by the quintuple $(\mathbf{A, B, C, D, x}_0)$ has the form $\mathcal{L}\{y\}(s) = \mathbf{H}_1\mathcal{L}\{u\}(s) + \mathbf{H}_2$ on some domain of convergence for complex variable s.

A term such as $\mathbf{H}_1 \triangleq \mathbf{C}(s\boldsymbol{I} - \mathbf{A})^{-1}\mathbf{B}$ or $\mathbf{H}_2 \triangleq \mathbf{C}(s\mathbf{I} - \mathbf{A})^{-1}\mathbf{x}_0$ is called a transfer matrix (or transfer function if scalar).

When suitably processed, the elements of $\mathbf{H}_1$ and $\mathbf{H}_2$ provide invariants used to form equations required for testing a LTI system for global *a priori* identifiability.

The worksheet defines procedures that can be called as required, making it applicable to LTI systems, such as Model 0 introduced in Chapter 1.

Introduction to Model 0: a LTI model

This worksheet considers an LTI structure for pyrolysis kinetics used to model the concentration of epimers of hopane found in oil-bearing rock (Whyte *et al.* (2002)).

The chemical network has reactions that convert precursors X and Y into products R and S respectively. R and S decay and mass is lost from the system. The network represents an open uncontrolled LTI compartmental structure:

$$\mathrm{X} \xrightarrow{k_3} \mathrm{R} \xrightarrow{k_7} \mathrm{D}_R$$

$$\mathrm{Y} \xrightarrow[k_4]{} \mathrm{S} \xrightarrow[k_8]{} \mathrm{D}_S$$

where k_3, k_4, k_7, k_8 are rate constants.

The state variables of the structure are $\boldsymbol{x}(t) = \begin{bmatrix} X(t) & Y(t) & R(t) & S(t) \end{bmatrix}^{\mathrm{T}}$ with initial state $\boldsymbol{x}(0) = \begin{bmatrix} X_0 & Y_0 & 0 & 0 \end{bmatrix}^{\mathrm{T}}$.

Experimental observations are given by $\boldsymbol{y}(t) = \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}\boldsymbol{x}(t)$.

The model has six strictly positive parameters, $\boldsymbol{\theta} = \begin{bmatrix} X_0 & Y_0 & k_3 & k_4 & k_7 & k_8 \end{bmatrix}^{\mathrm{T}}$.

Model 0 is uncontrolled and hence there is no $\mathbf{H}_1$ to consider. Note that in this case $\mathcal{L}\{y\} = \mathbf{H}_2$, and the problem of putting $\mathbf{H}_1$ and $\mathbf{H}_2$ into the canonical form reduces to

that for uncontrolled LTI systems. That is, we only have to ensure that $\mathcal{L}\{y\}$ is in the canonical form.

```
> version(version); interface(version);
 User Interface: 1097895
        Kernel: 1097895
       Library: 1097895
                                1097895
```

Standard Worksheet Interface, Maple 2015.2, Linux, December 21 2015 Build **(A1.1)**

ID 1097895

```
> restart: with(linalg): with(inttrans): with(Groebner):
```

Procedures used in processing test cases

Three procedures are defined here to automate some of the processing of transfer matrix elements and the formation of the identifiability equations.

A procedure for putting a transfer matrix into canonical form: process_matrix

This procedure takes a transfer matrix as input and ensures that the rational functions that are its elements are put into the canonical form.

The procedure uses the "normal" command to ensure that each rational function is relatively prime, and then expands both the numerator and denominator so that the coefficients of each polynomial are clearly associated with a particular monomial term. A warning is given for any rational function that does not have a monic denominator. A means for ensuring that the denominator is monic is not obvious in Maple, as dividing both numerator and denominator by the leading coefficient of the denominator does not change the display of the rational function, possibly to avoid coefficients that are themselves rational functions, in this case, in the parameters. For the particular structures we study in this thesis, the rational functions always have monic denominators.

The input "sort_order" is used in sorting each numerator and denominator: polynomials are sorted from highest to lowest power in the complex variable s and then each coefficient is sorted according to the lexicographical order of parameters specified in "sort_order". This is designed to give uniformity in how parameters appear in the coefficients.

```
> process_matrix:=proc(sort_order,transfer_matrix)
> local i:=0, j:=0, colMAX, rowMAX, latest,
  leading_denom_coeff, processed_matrix:=Matrix(rowdim
  (transfer_matrix),coldim(transfer_matrix));
>  rowMAX:=rowdim(transfer_matrix): colMAX:=coldim
  (transfer_matrix):
> for i from 1 to rowMAX do; for j from 1 to colMAX do;
```

```
> processed_matrix[i,j]:=normal(transfer_matrix[i,j]);
> leading_denom_coeff:= lcoeff(denom(processed_matrix[i,j]),s)
  ;  if (leading_denom_coeff <> 1) then print(`Note element`,
  i,j, `of this processed matrix has a denominator that is not
  monic`); fi;
> processed_matrix[i,j]:=sort(collect(numer(processed_matrix
  [i,j]),s),sort_order,plex) /sort(collect(denom
  (processed_matrix[i,j]),s),sort_order,plex); od; od;
> return(processed_matrix); end;
```

```
process_matrix := proc(sort_order, transfer_matrix)                    (A2.1.1)
    local i, j, colMAX, rowMAX, latest, leading_denom_coeff,
    processed_matrix;
    i := 0;
    j := 0;
    processed_matrix := Matrix(linalg:-rowdim(transfer_matrix), linalg:-
    coldim(transfer_matrix));
    rowMAX := linalg:-rowdim(transfer_matrix);
    colMAX := linalg:-coldim(transfer_matrix);
    for i to rowMAX do
        for j to colMAX do
            processed_matrix[i, j] := normal(transfer_matrix[i, j]);
            leading_denom_coeff := lcoeff(denom(processed_matrix[i, j
            ]), s);
            if leading_denom_coeff <> 1 then
                print(`Note element`, i, j,
                `of this processed matrix has a denominator that is not
                monic`)
            end if;
            processed_matrix[i, j] := sort(collect(numer(processed_matrix
            [i, j]), s), sort_order, plex)
            / sort(collect(denom(processed_matrix[i, j]), s), sort_order,
            plex)
        end do
    end do;
    return processed_matrix
```

```
end proc
```

A procedure for extracting coefficients from rational functions in a processed transfer matrix: collect_invariants

```
> collect_invariants:=proc(processed_matrix)
> local i:=0, j:=0, latest, coeff_set:={};
> for i from 1 to rowdim(processed_matrix) do; for j from 1 to
  coldim(processed_matrix) do;
> latest:={coeffs(expand(numer(processed_matrix[i,j])),s)}
  union {coeffs(expand(denom(processed_matrix[i,j])),s)};
  coeff_set:=coeff_set union latest;  od; od; return
  (coeff_set); end;
```

```
collect_invariants := proc(processed_matrix)                    (A2.2.1)
    local i, j, latest, coeff_set;
    i := 0;
    j := 0;
    coeff_set := { };
    for i to linalg:-rowdim(processed_matrix) do
        for j to linalg:-coldim(processed_matrix) do
            latest := {coeffs(expand(numer(processed_matrix[i, j])), s)}
            union {coeffs(expand(denom(processed_matrix[i, j])), s)};
            coeff_set := coeff_set union latest
        end do
    end do;
    return coeff_set
end proc
```

A procedure for forming identifiability equations: identifiability_eqn_list

The following procedure takes a vector of invariants obtained from a system, the vector of system parameters **θ**, and a vector of unspecified values of **θ** called **θ'** which may be different from the true value, and forms a list of equations used subsequently in testing the system for global *a priori* identifiability.

```
> identifiability_eqn_list:=proc(input_list,theta,theta_prime,
  eqn_list)
> local ii:=0, j:=0, latest, replacements:=[], new_list:=[];
> for j from 1 to nops(theta) do; latest:=theta[j]=theta_prime
```

```
  [j]; replacements:=[op(replacements),latest]; od;
> new_list:=eqn_list; for ii from 1 to nops(input_list) do;
> new_list:=[op(new_list),input_list[ii]=subs(replacements,
  input_list[ii]) ]; od; return(new_list); end;
```

identifiability_eqn_list := **proc**(*input_list*, theta, *theta_prime*, *eqn_list*) **(A2.3.1)**

local *ii*, *j*, *latest*, *replacements*, *new_list*;

ii := 0;

j := 0;

replacements := [ ];

new_list := [ ];

for *j* **to** *nops*(theta) **do**

latest := theta[*j*] = *theta_prime*[*j*];

replacements := [*op*(*replacements*), *latest*]

end do;

new_list := *eqn_list*;

for *ii* **to** *nops*(*input_list*) **do**

new_list := [*op*(*new_list*), *input_list*[*ii*] = *subs*(*replacements*, *input_list*[*ii*])]

end do;

return *new_list*

end proc

Consideration of Structure 0

Setting up the structure

The **A1** matrix describes the interconversions of chemical species believed to occur in an experiment.

```
> A1:=Matrix(4,4,[-k[3],0,0,0,0,-k[4],0,0,k[3],0,-k[7],0,0,k
  [4],0,-k[8] ]);
```

$$A1 := \begin{bmatrix} -k_3 & 0 & 0 & 0 \\ 0 & -k_4 & 0 & 0 \\ k_3 & 0 & -k_7 & 0 \\ 0 & k_4 & 0 & -k_8 \end{bmatrix} \quad \textbf{(A3.1.1)}$$

We have initial state

```
> x0_1:=Matrix(4,1,[X[0],Y[0],0,0]);
```

$$x0\_1 := \begin{bmatrix} X_0 \\ Y_0 \\ 0 \\ 0 \end{bmatrix} \quad \textbf{(A3.1.2)}$$

As Structure 0 is composed of uncontrolled systems,

```
> B1:=Matrix(4,1,[0,0]); D1:=Matrix(2,1,[0,0]);
```

$$B1 := \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

$$D1 := \begin{bmatrix} 0 \\ 0 \end{bmatrix} \quad \textbf{(A3.1.3)}$$

The observation gain matrix is

```
> C1:=Matrix(2,4,[0,0,1,0,0,0,0,1]);
```

$$C1 := \begin{bmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad \textbf{(A3.1.4)}$$

The vector of parameters $\boldsymbol{\theta}$ is

```
> theta:=[X[0],Y[0],k[3],k[4],k[7],k[8]];
```

$$\theta := [X_0, Y_0, k_3, k_4, k_7, k_8] \quad \textbf{(A3.1.5)}$$

which we mimic in defining the vector of alternative parameter values $\boldsymbol{\theta}'$ belonging to the same set as $\boldsymbol{\theta}$:

```
> theta_prime:=[chi[0],Upsilon[0],Kappa[3],Kappa[4],Kappa[7],
  Kappa[8]];
```

$$theta\_prime := [\chi_0, \Upsilon_0, K_3, K_4, K_7, K_8] \quad \textbf{(A3.1.6)}$$

Formation of transfer matrices and checking if their components are in the canonical form

Following the introduction, $\mathcal{L}\{y\} = \mathbf{H}_1\mathcal{L}\{u\} + \mathbf{H}_2$. To derive these matrices we start by forming $(sI - A_1)$

```
> Imat:=Matrix(4,shape=identity);
```

$$Imat := \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad \textbf{(A3.2.1)}$$

```
> prelim1:=Matrix(matadd(Imat,A1,s,-1)):
```
and inverting this allows us to calculate our transfer matrices
```
> H2:=Matrix(multiply(C1,inverse(prelim1),x0_1));
```

$$H2 := \begin{bmatrix} \frac{k_3 X_0}{(s+k_3)(s+k_7)} \\ \frac{k_4 Y_0}{(s+k_4)(s+k_8)} \end{bmatrix} \quad \textbf{(A3.2.2)}$$

```
> H1:=Matrix(matadd(multiply(C1,B1),D1));
```

$$H1 := \begin{bmatrix} 0 \\ 0 \end{bmatrix} \quad \textbf{(A3.2.3)}$$

We will process these matrices to obtain the desired form

```
> sort_order:=[s,X[0],Y[0],k[3],k[4],k[7],k[8]];
```

$$sort\_order := [s, X_0, Y_0, k_3, k_4, k_7, k_8] \quad \textbf{(A3.2.4)}$$

```
> H1_proc:=process_matrix(sort_order,H1);
```

$$H1\_proc := \begin{bmatrix} 0 \\ 0 \end{bmatrix} \quad \textbf{(A3.2.5)}$$

```
> H2_proc:=process_matrix(sort_order,H2);
```

$$H2\_proc := \begin{bmatrix} \frac{X_0 k_3}{s^2 + (k_3 + k_7) s + k_3 k_7} \\ \frac{Y_0 k_4}{s^2 + (k_4 + k_8) s + k_4 k_8} \end{bmatrix} \quad \textbf{(A3.2.6)}$$

and we can collect invariants from these matrices

Obtaining invariants from (A3.2.6) to form ϕ_1

The elements of ϕ_1 are the coefficients of the rational functions in canonical form obtained from matrices $\mathbf{H}_1$ and $\mathbf{H}_2$.

```
> H1_inv:=collect_invariants(H1_proc);
```

$$H1\_inv := \{0, 1\} \quad \textbf{(A3.3.1)}$$

```
> H2_inv:=collect_invariants(H2_proc);
```

$$H2\_inv := \{1, X_0 k_3, k_3 k_7, Y_0 k_4, k_4 k_8, k_3 + k_7, k_4 + k_8\} \quad \textbf{(A3.3.2)}$$

Collecting the coefficients gives

```
> coeff_collection:= H1_inv union H2_inv;
```

$$coeff\_collection := \{0, 1, X_0 k_3, k_3 k_7, Y_0 k_4, k_4 k_8, k_3 + k_7, k_4 + k_8\} \quad \textbf{(A3.3.3)}$$

Here take only the coefficients from **(A3.3.3)** that depend on the system parameters. This is achieved by excluding any elements that are numeric in type.

```
> phivec1:={}: for i from 1 to nops(coeff_collection) do; if
  (is(coeff_collection[i],numeric) = false) then phivec1:=
  phivec1 union {coeff_collection[i]}; fi; od;
```

```
> phivec1;
```

$$\{X_0\,k_3,\ k_3\,k_7,\ Y_0\,k_4,\ k_4\,k_8,\ k_3+k_7,\ k_4+k_8\} \qquad \textbf{(A3.3.4)}$$

```
> eqn_list:=[]; phi1vec_list:=convert(phivec1,list);
  new_list:=[];
```

$$eqn\_list := [\,]$$

$$phi1vec\_list := [X_0\,k_3,\ k_3\,k_7,\ Y_0\,k_4,\ k_4\,k_8,\ k_3+k_7,\ k_4+k_8]$$

$$new\_list := [\,] \qquad \textbf{(A3.3.5)}$$

We Call the subroutine identifiability_eqn_list with input ϕ_1 to generate the complete set of identifiability equations

```
> phase1_eqn_list:=identifiability_eqn_list(phi1vec_list,
  theta,theta_prime,new_list);
```

$$phase1\_eqn\_list := [X_0\,k_3 = \chi_0\,\mathrm{K}_3,\ k_3\,k_7 = \mathrm{K}_3\,\mathrm{K}_7,\ Y_0\,k_4 = \mathrm{Y}_0\,\mathrm{K}_4,\ k_4\,k_8 = \mathrm{K}_4\,\mathrm{K}_8, \qquad \textbf{(A3.3.6)}$$

$$k_3+k_7 = \mathrm{K}_3+\mathrm{K}_7,\ k_4+k_8 = \mathrm{K}_4+\mathrm{K}_8]$$

and solving the equations given in **(A3.3.6)** for **θ'** in terms of **θ** gives

```
> solset:=solve(phase1_eqn_list,theta_prime);
```

$$solset := \left[\left[\chi_0 = X_0,\ \mathrm{Y}_0 = Y_0,\ \mathrm{K}_3 = k_3,\ \mathrm{K}_4 = k_4,\ \mathrm{K}_7 = k_7,\ \mathrm{K}_8 = k_8\right],\ \left[\chi_0 = X_0,\ \mathrm{Y}_0 \right.\right. \qquad \textbf{(A3.3.7)}$$

$$= \frac{Y_0\,k_4}{k_8},\ \mathrm{K}_3 = k_3,\ \mathrm{K}_4 = k_8,\ \mathrm{K}_7 = k_7,\ \mathrm{K}_8 = k_4\Big],\ \left[\chi_0 = \frac{X_0\,k_3}{k_7},\ \mathrm{Y}_0 = Y_0,\ \mathrm{K}_3\right.$$

$$\left. = k_7,\ \mathrm{K}_4 = k_4,\ \mathrm{K}_7 = k_3,\ \mathrm{K}_8 = k_8\right],\ \left[\chi_0 = \frac{X_0\,k_3}{k_7},\ \mathrm{Y}_0 = \frac{Y_0\,k_4}{k_8},\ \mathrm{K}_3 = k_7,\ \mathrm{K}_4\right.$$

$$\left.\left. = k_8,\ \mathrm{K}_7 = k_3,\ \mathrm{K}_8 = k_4\right]\right]$$

The solution set **(A3.3.7)** shows that there are four distinct sets of solutions. Hence, Structure 0 is locally *a priori* identifiable but not globally *a priori* identifiable.

Appendix C

Maple analysis of Structure $\mathcal{M}$

In this worksheet we analyse the three-parameter form of the simple bimolecular model.

Introduction to the three-parameter form of the simple bimolecular model: Structure m

We derive the three-parameter form of the simple bimolecular model from the four-parameter form by considering the components of the response and removing that fixed component associated with the mass of immobilised ligand. Alternatively, we may view the three-parameter form as being obtained from the four-parameter version by a state transformation.

The chemical interaction is between one analyte molecule A and one immobilised ligand molecule B to form one complex (AB):

$\mathrm{A} + \mathrm{B} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} (\mathrm{AB})$ where k_a (k_d) is the forward (reverse) reaction rate constant

and the physical system has dynamics governed by the differential equations for molar concentrations

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{B}] = -k_a[\mathrm{A}](t)\cdot[\mathrm{B}] + k_d\cdot[(\mathrm{AB})],$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[(\mathrm{AB})] = k_a[\mathrm{A}](t)\cdot[\mathrm{B}] - k_d\cdot[(\mathrm{AB})],$$

where analyte concentration $[A](t) = \begin{cases} \alpha, & 0 \le t < t_1, \\ 0, & t \ge t_1, \end{cases}$ for switching time t_1.

The non-negative state vector has initial conditions $\left[\begin{array}{c}[\mathrm{B}]\\ [(\mathrm{AB})]\end{array}\right]\Big|_{t=0} = \left[\begin{array}{c}\beta_1\\ 0\end{array}\right]$.

We model experimental response by the scalar expression

$$y(t, \boldsymbol{\theta}) = [0, 1]\left[\begin{array}{c}[\mathrm{B}](\mathrm{t}, \boldsymbol{\theta})\\ [(\mathrm{AB})](\mathrm{t}, \boldsymbol{\theta})\end{array}\right],$$

where

$\boldsymbol{\theta} = \left[\begin{array}{ccc} k_a & k_d & \beta_1 \end{array}\right]^{\mathrm{T}}$ is the parameter vector having strictly positive elements.

These relations form the representative system of a structure of uncontrolled LSS of one transition, denoted by m.

LTI structures $m[1]$ and $m[2]$ are obtained from m as described in Chapter 3. Structure $m[i]$ (i=1,2) has state vector $\boldsymbol{x}^{[i]}$ and output $y^{[i]}$.

Preliminary comments: For the representative system of a controlled LTI structure

we have a general expression the Laplace transform of response *y*, $\mathcal{L}(y) = \mathbf{H}_1\mathcal{L}(\boldsymbol{u}) + \mathbf{H}_2$, in terms of transfer matrices
. The elements of these matrices are rational functions. In order to test the structure for global *a priori* identifiability we must ensure that each rational function is in the canonical form.

For LTI structures derived from a LSS structure, we adapt the general form of $\mathcal{L}(y)$ given above. Here, for a LSS structure m and the derived LTI structure $m^{[i]}$ (*i*=1,2) we have $\mathcal{L}(y^{[i]}) = \mathbf{H}_1{}^{[i]}\mathcal{L}(\boldsymbol{u}) + \mathbf{H}_2{}^{[i]}$.
Here we have that *structure* m is uncontrolled and has scalar output. Hence the appropriate $\mathbf{H}_2$ for each of the two representative LTI systems is zero and $\mathbf{H}_1$ is a scalar in each case.
Hence, $\mathcal{L}(y^{[i]}) = \mathbf{H}_2{}^{[i]}$, and so the problem of putting elements of $\mathbf{H}_1$ and $\mathbf{H}_2$ into canonical form reduces to the description of the problem given for uncontrolled LTI systems.
This is the problem of ensuring $\mathcal{L}(y^{[i]})$ is in the canonical form.

Maple Notation: Matrices or vectors belonging to or arising from the first or second LTI systems in effect are distinguished respectively by a 1 or 2 suffix or subscript.

```
> version(version); interface(version);
 User Interface: 1097895
        Kernel: 1097895
       Library: 1097895
                                1097895
```

Standard Worksheet Interface, Maple 2015.2, Linux, December 21 2015 Build **(B1.1)**

ID 1097895

```
> with(linalg): with(inttrans): with(Groebner):
```

Procedures used in processing test cases

Three procedures are defined here to automate some of the processing of transfer matrix elements and the formation of the equations for testing a structure for global *a priori* identifiability.
Sections containing subroutines are collapsed for brevity. Code for these is given in Appendix A.

A procedure for putting a transfer matrix into canonical form: process_matrix

A procedure for extracting coefficients from rational functions in a processed transfer matrix: collect_invariants

A procedure for forming identifiability equations:

identifiability_eqn_list

Consideration of $m^{[1]}$

Setting up $m^{[1]}$

The **A1** matrix describes the dynamics of the association phase of an experiment.

```
> A1:=Matrix(2,2,[-k[a]*alpha,k[d],k[a]*alpha,-k[d]]);
```

$$A1 := \begin{bmatrix} -\alpha\, k_a & k_d \\ \alpha\, k_a & -k_d \end{bmatrix} \quad \textbf{(B3.1.1)}$$

The state vector $x^{[1]}$ has initial value $x^{[1]}(0)$:

```
> x0_1:=Matrix(2,1,[beta[1],0]);
```

$$x0\_1 := \begin{bmatrix} \beta_1 \\ 0 \end{bmatrix} \quad \textbf{(B3.1.2)}$$

As m is an uncontrolled structure,

```
> B1:=Matrix(2,1,[0,0]); D1:=Matrix(1,1,[0]);
```

$$B1 := \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$D1 := \begin{bmatrix} 0 \end{bmatrix} \quad \textbf{(B3.1.3)}$$

The observation gain matrix is

```
> C1:=Matrix(1,2,[0,1]);
```

$$C1 := \begin{bmatrix} 0 & 1 \end{bmatrix} \quad \textbf{(B3.1.4)}$$

As all parameters in the LSS m are introduced in the LTI $m^{[1]}$, we can define the vector of parameters **θ**

```
> theta:=[k[a],k[d],beta[1]];
```

$$\theta := [k_a, k_d, \beta_1] \quad \textbf{(B3.1.5)}$$

and the vector of alternative parameter values **θ'** belonging to the same set as **θ**

```
> theta_prime:=[Kappa[a],Kappa[d],b[1]];
```

$$theta\_prime := [\mathrm{K}_a, \mathrm{K}_d, b_1] \quad \textbf{(B3.1.6)}$$

Formation of transfer matrices and checking if their components are in the canonical form

Following the introduction, $\mathcal{L}(y^{[1]}) = \mathbf{H}_2^{[1]}$. To derive this matrix, start by

forming $(sI - A_1)$.

```
> prelim1:=Matrix(matadd(Matrix(2,shape=identity),A1,s,-1)):
```

and invert to obtain the desired matrix:

```
> H2_1:=Matrix(multiply(C1,inverse(prelim1),x0_1));
```

$$H2\_1 := \left[\frac{k_a \alpha \beta_1}{s(\alpha k_a + s + k_d)} \right] \quad \textbf{(B3.2.1)}$$

We will sort expressions using the following ordering:

```
> sort_order:=[s,k[a],A,k[d],beta[1]];
```

$$sort\_order := [s, k_a, A, k_d, \beta_1] \quad \textbf{(B3.2.2)}$$

which is applied after placing rational functions in the canonical form

```
> H2_1_proc:=process_matrix(sort_order,H2_1);
```

$$H2\_1\_proc := \left[\frac{\alpha k_a \beta_1}{s^2 + (\alpha k_a + k_d)\, s} \right] \quad \textbf{(B3.2.3)}$$

Obtaining the invariants from (B3.2.1) to form $\phi^{[1]}$

The elements of $\phi^{[1]}$ are the coefficients of the rational functions in canonical form obtained from $\mathbf{H}_2^{[1]}$.

```
> H2_1_inv:=collect_invariants(H2_1_proc);
```

$$H2\_1\_inv := \{1, \alpha k_a \beta_1, \alpha k_a + k_d\} \quad \textbf{(B3.3.1)}$$

```
> coeff_collection:= H2_1_inv;
```

$$coeff\_collection := \{1, \alpha k_a \beta_1, \alpha k_a + k_d\} \quad \textbf{(B3.3.2)}$$

Only the coefficients from **(B3.3.2)** that depend on the parameters are useful. We obtain these from the set above by excluding any elements that are numeric.

```
> phivec1:={}: for i from 1 to nops(coeff_collection) do; if
  (is(coeff_collection[i],numeric) = false) then phivec1:=
  phivec1 union {coeff_collection[i]}; fi; od;
> phivec1;
```

$$\{\alpha k_a \beta_1, \alpha k_a + k_d\} \quad \textbf{(B3.3.3)}$$

```
> eqn_list:=[]; phi1vec_list:=convert(phivec1,list);
  new_list:=[];
```

$$eqn\_list := [\,]$$

$$phi1vec\_list := [\alpha k_a \beta_1, \alpha k_a + k_d]$$

$$new\_list := [\,] \quad \textbf{(B3.3.4)}$$

Call the subroutine identifiability_eqn_list with input $\phi^{[1]}$ to generate the set of

identifiability equations available thus far

```
> phase1_eqn_list:=identifiability_eqn_list(phi1vec_list,
  theta,theta_prime,new_list);
```

$$phase1\_eqn\_list := \left[\alpha\, k_a \beta_1 = \alpha\, \mathrm{K}_a\, b_1,\ \alpha\, k_a + k_d = \alpha\, \mathrm{K}_a + \mathrm{K}_d\right] \quad \textbf{(B3.3.5)}$$

Solving the equations given in **(B3.3.5)** for $\boldsymbol{\theta}'$ in terms of $\boldsymbol{\theta}$ gives

```
> solset:=solve(phase1_eqn_list,theta_prime);
```

$$solset := \left[\left[\mathrm{K}_a = \frac{\beta_1\, k_a}{b_1},\ \mathrm{K}_d = \frac{\alpha\, b_1\, k_a - \alpha\, \beta_1\, k_a + b_1\, k_d}{b_1},\ b_1 = b_1\right]\right] \quad \textbf{(B3.3.6)}$$

The solution set **(B3.3.6)** shows that b_1 is a free parameter and hence that there are multiple solutions for K_a and K_d. This is sufficient for us to decide that m cannot be classified as globally *a priori* identifiable using only the parameter information obtainable from $m^{[1]}$. It is necessary to proceed to consider the information obtainable from $m^{[2]}$.

Consideration of $m^{[2]}$

Setting up $m^{[2]}$

The state vector of $m^{[2]}(\boldsymbol{\theta})$ has initial conditions determined by the state reached by $m^{[1]}(\boldsymbol{\theta})$ immediately before the start of the dissociation phase, that is, at time t_1.

The **A2** matrix represents the dynamics of the dissociation phase of an experiment,

```
> A2:=Matrix(2,2,[0,k[d],0,-k[d]]);
```

$$A2 := \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix} \quad \textbf{(B4.1.1)}$$

The initial state of $m^{[2]}(\boldsymbol{\theta})$, $\boldsymbol{x}^{[2]}(\mathbf{0}, \boldsymbol{\theta})$, is

```
> x0_2:=Matrix(2,1,[x0[2,1],x0[2,2]]);
```

$$x0\_2 := \begin{bmatrix} x0_{2,1} \\ x0_{2,2} \end{bmatrix} \quad \textbf{(B4.1.2)}$$

With no changes to the experimental conditions other than the analyte concentration, these matrices are equal to their counterparts in $m^{[1]}$

```
> B2:=Matrix(B1); C2:=Matrix(C1); D2:=Matrix(D1);
```

$$B2 := \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$C2 := \begin{bmatrix} 0 & 1 \end{bmatrix}$$

$$D2 := \begin{bmatrix} 0 \end{bmatrix} \quad \textbf{(B4.1.3)}$$

Formation of the transfer matrices of $m^{[2]}$

```
> prelim2:=Matrix(matadd(Matrix(2,shape=identity),A2,s,-1)):
> H2_2:=Matrix(multiply(C2,inverse(prelim2),x0_2));
```

$$H2\_2 := \begin{bmatrix} \frac{x0_{2,2}}{s + k_d} \end{bmatrix} \quad \textbf{(B4.2.1)}$$

Analogously to the previous phase, $\mathcal{L}(y^{[2]}) = \mathbf{H}_2{}^{[2]}$ given by **(B4.2.1)** is clearly in the canonical form as the one rational function present has a monic denominator and is a constant over a linear function.
Hence, cancellation of the factor $(s + k_d)$ is not possible.

Collecting invariants from (B4.2.1) to form $\phi^{[2]}$ and extracting those components which are useful

```
> phivector2:=convert(collect_invariants(H2_2),list);
```

$$phivector2 := [1, k_d, x0_{2,2}] \quad \textbf{(B4.3.1)}$$

The term in **(B4.3.1)** that depends on the initial state of $m^{[2]}$ is not useful for the proposed identifiability test. Let us exclude this and only obtain the invariant from $m^{[2]}$ that we can use directly.
That is, rather than solve $m^{[1]}(\boldsymbol{\theta})$ to obtain an explicit expression for $\boldsymbol{x}^{[2]}(0, \boldsymbol{\theta}) = \boldsymbol{x}^{[1]}(t_1, \boldsymbol{\theta})$, let us consider the information we can obtain from $m^{[2]}$ without such an expression.

```
> reduced_coeff:=[]: excluded:=[]:
> for i from 1 to nops(phivector2) do;  if (diff(phivector2
  [i],x0[2,2]) =0 and is(phivector2[i],numeric) = false)
  then reduced_coeff:=[op(reduced_coeff),phivector2[i]] else
  excluded:=[op(excluded),phivector2[i]] fi; od;
> print(reduced_coeff); print(excluded);
```

$$[k_d]$$

$$[1, x0_{2,2}] \quad \textbf{(B4.3.2)}$$

```
> usable_invariants:=[op(phivec1),op(reduced_coeff)];
```

$$usable\_invariants := [\alpha\, k_a \beta_1, \alpha\, k_a + k_d, k_d] \quad \textbf{(B4.3.3)}$$

Testing m for global *a priori* identifiability

The identifiability equations that do not depend on $\mathbf{x}^{[2]}(0)$ are formed from **(B4.3.3)**:

```
> usable_eqn_list:=identifiability_eqn_list(reduced_coeff,theta,
  theta_prime,phase1_eqn_list);
```

$$usable\_eqn\_list := \left[\alpha\, k_a \beta_1 = \alpha\, \mathrm{K}_a\, b_1,\ \alpha\, k_a + k_d = \alpha\, \mathrm{K}_a + \mathrm{K}_d,\ k_d = \mathrm{K}_d\right] \quad \textbf{(B5.1)}$$

```
> s2:=solve(usable_eqn_list,theta_prime);
```

$$s2 := \left[\left[\mathrm{K}_a = k_a,\ \mathrm{K}_d = k_d,\ b_1 = \beta_1\right]\right] \quad \textbf{(B5.2)}$$

The solution set **(B5.2)** shows that the error-free and infinite output of $m(\boldsymbol{\theta}')$ is only equal to that of $m(\boldsymbol{\theta})$ (where $\boldsymbol{\theta}$ is any feasible parameter value) if $\boldsymbol{\theta}' = \boldsymbol{\theta}$. That is, m is globally *a priori* identifiable.

Appendix D

Maple analysis of Structure $\mathcal{N}$

This worksheet analyses a four-parameter form of the simple bimolecular model.

Introduction to the four-parameter form of the simple bimolecular model: Structure η

The chemical interaction is between one analyte molecule A and one immobilised ligand molecule B to form one complex (AB):

A + B $\underset{k_d}{\overset{k_a}{\rightleftharpoons}}$ (AB) where k_a (k_d) is the forward (reverse) reaction rate constant.

The physical system has dynamics governed by the differential equations for molar concentrations

$$\frac{d}{dt}[B] = -k_a[A](t)\cdot[B] + k_d\cdot[(AB)],$$

$$\frac{d}{dt}[(AB)] = k_a[A](t)\cdot[B] - k_d\cdot[(AB)],$$

where analyte concentration $[A](t) = \begin{cases} \alpha, & 0 \le t < t_1, \\ 0, & t \ge t_1, \end{cases}$ for switching time t_1.

The non-negative state vector has initial conditions $\left[\begin{array}{c}[B]\\ [(AB)]\end{array}\right]\Big|_{t=0} = \left[\begin{array}{c}B_0\\ 0\end{array}\right]$.

We model experimental response by the scalar expression

$y(t, \boldsymbol{\theta}) = [0, \rho_A]\left[\begin{array}{c}[B](t, \boldsymbol{\theta})\\ [(AB)](t, \boldsymbol{\theta})\end{array}\right]$, where the parameter vector

$\boldsymbol{\theta} = \left[\begin{array}{cccc} k_a & k_d & B_0 & \rho_A \end{array}\right]$ has four strictly positive parameters.

These relations comprise system $\eta(\boldsymbol{\theta})$, the representative system of η, a structure of uncontrolled LSS of one transition (ULSS-1).

We obtain LTI structures $\eta^{[1]}$ and $\eta^{[2]}$ from η as described in Chapter 3.

The preliminary notes made in the worksheet that analysed structure M also apply here.

Maple Notation: Matrices or vectors belonging to or arising from the first or second LTI systems in effect are distinguished respectively by a 1 or 2 suffix or subscript.

```
> version(version); interface(version);
 User Interface: 1097895
        Kernel: 1097895
       Library: 1097895
                                1097895
```

Standard Worksheet Interface, Maple 2015.2, Linux, December 21 2015 Build **(C1.1)**
ID 1097895

```
> with(linalg): with(inttrans):
```

Procedures used in processing test cases

Three procedures are defined here to automate some of the processing of transfer matrix elements and the formation of the identifiability equations.
Sections containing subroutines are collapsed for brevity. The code is given in Appendix A.

A procedure for putting a transfer matrix into canonical form: process_matrix

A procedure for extracting coefficients from rational functions in a processed transfer matrix: collect_invariants

A procedure for forming identifiability equations: identifiability_eqn_list

Consideration of $n^{[1]}$

Setting up $n^{[1]}$

The **A1** matrix describes the dynamics of the association phase of an experiment.

```
> A1:=Matrix(2,2,[-k[a]*alpha,k[d],k[a]*alpha,-k[d]]);
```

$$A1 := \begin{bmatrix} -k_a \alpha & k_d \\ k_a \alpha & -k_d \end{bmatrix} \quad \textbf{(C3.1.1)}$$

Initial conditions for the state vector $x^{[1]}(\cdot, \theta)$, $x^{[1]}(0, \theta)$, are

```
> x0_1:=Matrix(2,1,[B[0],0]);
```

$$x0\_1 := \begin{bmatrix} B_0 \\ 0 \end{bmatrix} \quad \textbf{(C3.1.2)}$$

As n is an uncontrolled structure, in terms of the general (controlled) LTI structure, some matrices are null:

```
> B1:=Matrix(2,1,[0,0]); D1:=Matrix(1,1,[0]);
```

$$B1 := \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$D1 := \begin{bmatrix} 0 \end{bmatrix} \quad \textbf{(C3.1.3)}$$

The observation gain matrix is

```
> C1:=Matrix(1,2,[0,rho[A]]);
```

$$C1 := \begin{bmatrix} 0 & \rho_A \end{bmatrix} \quad \textbf{(C3.1.4)}$$

As all parameters in $\mathcal{N}(\boldsymbol{\theta})$ are introduced in $\mathcal{N}^{[1]}(\boldsymbol{\theta})$, we can define the vector of parameters $\boldsymbol{\theta}$

```
> theta:=[k[a],k[d],rho[A],B[0]];
```

$$\theta := [k_a, k_d, \rho_A, B_0] \quad \textbf{(C3.1.5)}$$

and the vector of alternative parameter values $\boldsymbol{\theta}'$ belonging to the same set as $\boldsymbol{\theta}$

```
> theta_prime:=[Kappa[a],Kappa[d],Rho[A],b[0]];
```

$$theta\_prime := [K_a, K_d, P_A, b_0] \quad \textbf{(C3.1.6)}$$

Formation of transfer matrix and checking if its components are in the canonical form

Following the introduction, $\mathcal{L}(y^{[1]}) = \mathbf{H}_2^{[1]}$. To derive this matrix, we start by forming $(sI - A_1)$.

```
> prelim1:=Matrix(matadd(Matrix(2,shape=identity),A1,s,-1)):
```

which we invert to obtain the desired matrix:

```
> H2_1:=Matrix(multiply(C1,inverse(prelim1),x0_1));
```

$$H2\_1 := \begin{bmatrix} \dfrac{\rho_A k_a \alpha B_0}{s(\alpha k_a + s + k_d)} \end{bmatrix} \quad \textbf{(C3.2.1)}$$

We will sort expressions using the following ordering

```
> sort_order:=[s,k[a],alpha,k[d],rho[A],B[0]];
```

$$sort\_order := [s, k_a, \alpha, k_d, \rho_A, B_0] \quad \textbf{(C3.2.2)}$$

which we apply after placing rational functions in the canonical form

```
> H2_1_proc:=process_matrix(sort_order,H2_1);
```

$$H2\_1\_proc := \begin{bmatrix} \dfrac{\alpha k_a \rho_A B_0}{s^2 + (k_a \alpha + k_d) s} \end{bmatrix} \quad \textbf{(C3.2.3)}$$

Obtaining invariants from (C3.2.3) to form $\boldsymbol{\phi}^{[1]}$

The elements of $\boldsymbol{\phi}^{[1]}$ are the coefficients of the rational functions in canonical form obtained from $\mathbf{H}_2^{[1]}$ (as presented in **(C3.2.3)**)

```
> coeff_collection:=collect_invariants(H2_1_proc);
```

(C3.3.1)

$$coeff\_collection := \{1,\ k_a\,\alpha\,\rho_A\,B_0,\ k_a\,\alpha + k_d\} \qquad \textbf{(C3.3.1)}$$

Only the coefficients from **(C3.3.1)** that depend on parameters are useful. Hence, we exclude any numeric elements.

```
> phivec1:={}: for i from 1 to nops(coeff_collection) do; if
  (is(coeff_collection[i],numeric) = false) then phivec1:=
  phivec1 union {coeff_collection[i]}; fi; od;
```

```
> phivec1;
```

$$\{k_a\,\alpha\,\rho_A\,B_0,\ k_a\,\alpha + k_d\} \qquad \textbf{(C3.3.2)}$$

```
> eqn_list:=[]; phi1vec_list:=convert(phivec1,list);
  new_list:=[];
```

$$eqn\_list := [\ ]$$

$$phi1vec\_list := [k_a\,\alpha\,\rho_A\,B_0,\ k_a\,\alpha + k_d]$$

$$new\_list := [\ ] \qquad \textbf{(C3.3.3)}$$

Call the subroutine identifiability_eqn_list with input $\boldsymbol{\phi}^{[1]}$ to generate the set of identifiability equations available thus far

```
> phase1_eqn_list:=identifiability_eqn_list(phi1vec_list,
  theta,theta_prime,new_list);
```

$$phase1\_eqn\_list := [k_a\,\alpha\,\rho_A\,B_0 = \mathrm{K}_a\,\alpha\,\mathrm{P}_A\,b_0,\ k_a\,\alpha + k_d = \alpha\,\mathrm{K}_a + \mathrm{K}_d] \qquad \textbf{(C3.3.4)}$$

Solving the equations given in **(C3.3.4)** for $\boldsymbol{\theta}'$ in terms of $\boldsymbol{\theta}$ gives

```
> solset:=solve(phase1_eqn_list,theta_prime);
```

$$solset := \left[\left[\mathrm{K}_a = \frac{\rho_A\,k_a\,B_0}{\mathrm{P}_A\,b_0},\ \mathrm{K}_d = \frac{-\alpha\,B_0\,k_a\,\rho_A + \alpha\,\mathrm{P}_A\,b_0\,k_a + \mathrm{P}_A\,b_0\,k_d}{\mathrm{P}_A\,b_0},\ \mathrm{P}_A = \mathrm{P}_A,\ b_0 = b_0\right]\right] \qquad \textbf{(C3.3.5)}$$

The solution set **(C3.3.5)** shows that b_0 and P_A are free parameters and hence that there are multiple solutions for K_a and K_d. As a result, we cannot classify n as globally *a priori* identifiable using only the parameter information obtainable from $n^{[1]}$. We proceed to consider the information obtainable from $n^{[2]}$.

Consideration of $n^{[2]}$

Setting up $n^{[2]}$

The LTI system $n^{[2]}(\boldsymbol{\theta})$ has initial state determined by the state reached by $n^{[1]}(\boldsymbol{\theta})$ immediately before the start of the dissociation phase, that is, at time t_1. Rather than solve $n^{[1]}(\boldsymbol{\theta})$ to obtain an explicit expression for

the initial state $\boldsymbol{x}^{[2]}(\mathbf{0}, \boldsymbol{\theta}) = \boldsymbol{x}^{[1]}(t_1, \boldsymbol{\theta})$, let us consider the information that is obtainable from $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ without such an expression. As $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ is a closed system, we have a conservation of mass constraint ${x^{[2]}}_1(0, \boldsymbol{\theta}) + {x^{[2]}}_2(0, \boldsymbol{\theta}) = B_0$.

The **A 2** matrix represents the dynamics of the dissociation phase of an experiment,

```
> A2:=Matrix(2,2,[0,k[d],0,-k[d]]);
```

$$A2 := \begin{bmatrix} 0 & k_d \\ 0 & -k_d \end{bmatrix} \qquad \textbf{(C4.1.1)}$$

Using the conservation of mass condition, we express the initial state of $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ as

```
> x0_2:=Matrix(2,1,[x0[2,1],x0[2,2]]);
```

$$x0\_2 := \begin{bmatrix} x0_{2,1} \\ x0_{2,2} \end{bmatrix} \qquad \textbf{(C4.1.2)}$$

Apart from a change to the the analyte concentration in the second phase, experimental conditions are the same as those used in the first phase. Hence some matrices of $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ are equal to their counterparts in $\mathcal{N}^{[1]}(\boldsymbol{\theta})$

```
> B2:=Matrix(B1); C2:=Matrix(C1); D2:=Matrix(D1);
```

$$B2 := \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$C2 := \begin{bmatrix} 0 & \rho_A \end{bmatrix}$$

$$D2 := \begin{bmatrix} 0 \end{bmatrix} \qquad \textbf{(C4.1.3)}$$

Formation of the transfer matrix of $\mathcal{N}^{[2]}$

```
> prelim2:=Matrix(matadd(Matrix(2,shape=identity),A2,s,-1)):
> H2_2:=Matrix(multiply(C2,inverse(prelim2),x0_2));
```

$$H2\_2 := \begin{bmatrix} \dfrac{\rho_A x0_{2,2}}{s + k_d} \end{bmatrix} \qquad \textbf{(C4.2.1)}$$

In this specific case $\mathcal{L}\left(y^{[2]}\right) = \mathbf{H}_2{}^{[2]}$ given by **(C4.2.1)** is clearly in the canonical form as the one rational function present has a monic denominator and is a constant over a linear function.
Hence, cancellation of the factor $(s + k_d)$ is not possible.

Collecting invariants from (C4.2.1) to form $\boldsymbol{\phi}^{[2]}$ and extracting those

components which are useful

```
> phivector2:=convert(collect_invariants(H2_2),list);
```

$$phivector2 := \left[1,\ k_d,\ \rho_A\, x0_{2,2}\right] \qquad \textbf{(C4.3.1)}$$

Terms in **(C4.3.1)** that depend on the initial state of $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ are not useful for the proposed identifiability test. Let us exclude these and only obtain the invariants from $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ that we can use directly. That is, rather than solve $\mathcal{N}^{[2]}(\boldsymbol{\theta})$ to obtain an explicit expression for $\boldsymbol{x}^{[2]}(0, \boldsymbol{\theta}) = \boldsymbol{x}^{[1]}(t_1, \boldsymbol{\theta})$, let us consider the information we can obtain from $\mathcal{N}^{[2]}$ without such an expression.

```
> reduced_coeff:=[]: excluded:=[]:
> for i from 1 to nops(phivector2) do;  if (diff(phivector2
  [i],x0[2,2]) = 0 and is(phivector2[i],numeric) = false)
  then reduced_coeff:=[op(reduced_coeff),phivector2[i]] else
  excluded:=[op(excluded),phivector2[i]] fi; od;
> print("useful",reduced_coeff); print("excluded",excluded);
```

$$\text{"useful"},\ \left[k_d\right]$$

$$\text{"excluded"},\ \left[1,\ \rho_A\, x0_{2,2}\right] \qquad \textbf{(C4.3.2)}$$

We combine the useful invariant above with those of $\boldsymbol{\phi}^{[1]}$ to form the vector of invariants we will use to creat the *a priori* identifiability test

```
> usable_invariants:=[op(phivec1),op(reduced_coeff)];
```

$$usable\_invariants := \left[k_a\,\alpha\,\rho_A\,B_0,\ k_a\,\alpha + k_d,\ k_d\right] \qquad \textbf{(C4.3.3)}$$

Forming equations and testing $\mathcal{N}$ for global *a priori* identifiability

We form the identifiability equations that do not depend on $\boldsymbol{x}^{[2]}(0)$ by using the invariants in **(C4.3.3)**

```
> usable_eqn_list:=identifiability_eqn_list(reduced_coeff,theta,
  theta_prime,phase1_eqn_list);
```

$$usable\_eqn\_list := \left[k_a\,\alpha\,\rho_A\,B_0 = \mathrm{K}_a\,\alpha\,\mathrm{P}_A\,b_0,\ k_a\,\alpha + k_d = \alpha\,\mathrm{K}_a + \mathrm{K}_d,\ k_d = \mathrm{K}_d\right] \qquad \textbf{(C5.1)}$$

and solving these gives

```
> s2:=solve(usable_eqn_list,theta_prime);
```

$$s2 := \left[\left[\mathrm{K}_a = k_a,\ \mathrm{K}_d = k_d,\ \mathrm{P}_A = \frac{\rho_A\,B_0}{b_0},\ b_0 = b_0\right]\right] \qquad \textbf{(C5.2)}$$

The solution set **(C5.2)** shows that $\boldsymbol{\theta}'$ is constrained such that its elements for the rate constant parameters can only reproduce the output of $\mathcal{N}$ with parameter $\boldsymbol{\theta}$ (any feasible parameter value) if $\mathrm{K}_a = k_a$ and $\mathrm{K}_d = k_d$. That is, these parameters must take the true value. We conclude that the rate constants are globally *a priori* identifiable.

(C5.2) also shows that b_0 is a free parameter. Considering this along with the

condition on P_A, we obtain $P_A b_0 = \rho_A B_0$, showing that there is an uncountably infinite set of values $\boldsymbol{\theta}'$ for which $\mathcal{N}(\boldsymbol{\theta}')$ can reproduce the output of $\mathcal{N}(\boldsymbol{\theta})$.

As a result of this feature, we cannot classify N as globally *a priori* identifiable using incomplete information (that is, using only the easily accessed invariants). However, we suspect that $P_A b_0 = \rho_A B_0$ indicates an unidentifiable structre. We demonstrate the validity of this suspicion with an *ad hoc* argument in Chapter 5.

Appendix E

Maple analysis of Structure $\mathcal{C}$

Third flow-cell biosensor model structure tested for global *a priori* identifiability: A structure of uncontrolled linear switching systems of one transition (ULSS-1), structure $\mathcal{C}$: the "two state conformational change model".

1 Introduction

This interaction is summarised by a reaction scheme relating analyte A, ligand B, complex (AB) and an alternative form of this complex, (AB)*:

k_a k_2 forward reaction rate constants

A + B $\rightleftharpoons$ (AB) $\rightleftharpoons$ (AB)*

k_d k_{-2} reverse reaction rate constants

The system has initial state $\left([B], [(AB)], [(AB)^*]\right)\Big|_{t=0} = (\beta_1, 0, 0)$.

Forming the parameter vector $\boldsymbol{\theta} = (k_a, k_d, k_2, k_{-2}, \beta_1)$,

the differential equation system for the state variables is

$$\frac{d}{dt}[B](t,\boldsymbol{\theta}) = -k_a \cdot [A(t)] \cdot [B](t,\boldsymbol{\theta}) + k_d \cdot [(AB)](t,\boldsymbol{\theta})$$

$$\frac{d}{dt}[(AB)](t,\boldsymbol{\theta}) = k_a \cdot [A(t)] \cdot [B](t,\boldsymbol{\theta}) - (k_d + k_2) \cdot [(AB)](t,\boldsymbol{\theta}) + k_{-2} \cdot [(AB)^*](t,\boldsymbol{\theta})$$

$$\frac{d}{dt}[(AB)^*](t,\boldsymbol{\theta}) = k_2 \cdot [(AB)](t,\boldsymbol{\theta}) - k_{-2} \cdot [(AB)^*](t,\boldsymbol{\theta})$$

with analyte concentration $[A(t)] = \begin{cases} \alpha_1 & 0 \le t < t_{1,} \\ 0 & t \ge t_{1,} \end{cases}$

where t_1 is the switching time.

By definition, rate constants k_a, k_d, k_2 and k_{-2} are positive. The initial amount of functional free ligand present in the system, β_1, is non-negative. However, as no reaction can proceed for $\beta_1 = 0$, we assume that β_1 is positive.

We model the response by $y(t,\boldsymbol{\theta}) = \begin{bmatrix} 0 & 1 & 1 \end{bmatrix} \begin{bmatrix} [B](t,\boldsymbol{\theta}) \\ [(AB)](t,\boldsymbol{\theta}) \\ [(AB)^*](t,\boldsymbol{\theta}) \end{bmatrix}$.

Let us use these relationships to compose the system $\mathcal{C}(\boldsymbol{\theta})$, the representative system of structure C.

Following the approach given in Chapter 3, ULSS-1 $\mathcal{C}(\boldsymbol{\theta})$ gives rise to two linear time-invariant systems.

The behaviour of the ULSS-1 prior to t_1 (the association phase of an experiment) is encapsulated by $\mathcal{C}^{[1]}$ having state vector $\boldsymbol{x}^{[1]}$, initial state $\boldsymbol{x}_0^{[1]}$ and output $y^{[1]}$.

The behaviour of the ULSS-1 from t_1 onwards (the dissociation phase) is given by $\mathcal{C}^{[2]}$ having state vector $\boldsymbol{x}^{[2]}$, initial state $\boldsymbol{x}_0^{[2]}$ and output $y^{[2]}$.

This worksheet explores methods aimed at classifying the structure, and shows that the results obtained by the SCUII algorithm agree with those from the SCReMI algorithm.

```
> version(version); interface(version); print("default number of
  digits used in calculations", Digits);
```

```
 User Interface: 1097895
        Kernel: 1097895
       Library: 1097895
                                1097895
```

Standard Worksheet Interface, Maple 2015.2, Linux, December 21 2015 Build ID 1097895

"default number of digits used in calculations", 10 **(E1.1)**

```
> with(LinearAlgebra): with(Optimization): with(VectorCalculus):
```

2 Routines to aid simplification of expressions

A routine for simplifying rational functions and collecting terms

```
> rat_fn_simplify:= proc(expr,sortlist)
  local result;
  result:= collect(numer(expr),sortlist)/collect(denom(expr),
  sortlist);
  return(result); end proc;
```

rat_fn_simplify := **proc**(*expr*, *sortlist*) **(E2.1)**

local *result*;

result := *collect*(*numer*(*expr*), *sortlist*) * 1 / *collect*(*denom*(*expr*), *sortlist*);

return *result*

end proc

A routine for collecting radicals (with the aim of simplifying expressions) as this is not achieved by the inbuilt Maple 16.02 commands:

```
> collect_radicals:=proc(expression,radicand_list,sortlist)
  # This proceedure takes a radicand and makes a substitution to
  enable a simplification, then
  # replaces the original expression.
  local i, Z, eqn_sub_set, reverse_reln, new_sortlist, new_exp,
  new_exp_final;
  # add the substitution variable to the start of the list of
  parameters for the sorting operation.
  Z:=convert(Vector(nops(radicand_list),symbol=Z),list);
  new_sortlist:=[op(Z),op(sortlist)];
  # Replace the radical term i with Z[i]^2 such that the square
```

```
  root term is simplified such that usual collection
  # routines work to sort and collect terms.
  eqn_sub_set:=[]; for i from 1 to nops(radicand_list) do;
  eqn_sub_set:=[op(eqn_sub_set),radicand_list[i]=Z[i]^2]; od;
  new_exp:=simplify(simplify(expression,eqn_sub_set),symbolic);
  # simplify the expression
  new_exp:=collect(sort(simplify(new_exp,radical, symbolic),
  new_sortlist),new_sortlist);
  # replace the artificial variable with the radicand in terms of
  the original parameters.
  reverse_reln:=[];
  for i from 1 to nops(radicand_list) do; reverse_reln:=[op
  (reverse_reln),Z[i]=sqrt(radicand_list[i])]; od;
  new_exp_final:=new_exp;
  for i from 1 to nops(radicand_list) do; new_exp_final:=subs
  (reverse_reln[i],new_exp_final); od;
  return(new_exp_final);
> end proc;
```

$$collect\_radicals := \mathbf{proc}(expression, radicand\_list, sortlist) \qquad \textbf{(E2.2)}$$

local $i, Z, eqn\_sub\_set, reverse\_reln, new\_sortlist, new\_exp, new\_exp\_final$;

$Z := convert(VectorCalculus:-Vector(nops(radicand\_list), symbol = Z),$
$list)$;

$new\_sortlist := [op(Z), op(sortlist)]$;

$eqn\_sub\_set := [\,]$;

for i **to** $nops(radicand\_list)$ **do**

$eqn\_sub\_set := [op(eqn\_sub\_set), radicand\_list[i] = Z[i]\text{^}2]$

end do;

$new\_exp := simplify(simplify(expression, eqn\_sub\_set), symbolic)$;

$new\_exp := collect(sort(simplify(new\_exp, radical, symbolic),$
$new\_sortlist), new\_sortlist)$;

$reverse\_reln := [\,]$;

for i **to** $nops(radicand\_list)$ **do**

$reverse\_reln := [op(reverse\_reln), Z[i] = \mathrm{sqrt}(radicand\_list[i])]$

end do;

$new\_exp\_final := new\_exp$;

for i **to** $nops(radicand\_list)$ **do**

$new\_exp\_final := subs(reverse\_reln[i], new\_exp\_final)$

end do;

```
    return new_exp_final
end proc
```

3 Consideration of structure $\mathcal{C}^{[1]}$

Setting up a general system in $\mathcal{C}^{[1]}$

```
> A1 := Matrix(3, 3, [-k[a]*alpha[1], k[d], 0, k[a]*alpha[1],
  -k[d]-k[2], k[-2], 0, k[2], -k[-2]]);
```

$$A1 := \begin{bmatrix} -k_a \alpha_1 & k_d & 0 \\ k_a \alpha_1 & -k_d - k_2 & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix} \quad \text{(E3.1.1)}$$

```
> x0_1 := Matrix(3, 1, [beta[1], 0, 0]);
```

$$x0\_1 := \begin{bmatrix} \beta_1 \\ 0 \\ 0 \end{bmatrix} \quad \text{(E3.1.2)}$$

```
> C1 := Matrix(1, 3, [0, 1, 1]);
```

$$C1 := \begin{bmatrix} 0 & 1 & 1 \end{bmatrix} \quad \text{(E3.1.3)}$$

The list that follows specifies all of the parameters that appear in the model structure $\mathcal{C}$.

```
> theta:=[beta[1],k[a],k[d],k[2],k[-2]];
```

$$\theta := [\beta_1, k_a, k_d, k_2, k_{-2}] \quad \text{(E3.1.4)}$$

`sortlist' dictates the ordering of parameters when sorting operations are used on algebraic expressions subsequently.

```
> sortlist:= [s,alpha[1],op(theta)];
```

$$sortlist := [s, \alpha_1, \beta_1, k_a, k_d, k_2, k_{-2}] \quad \text{(E3.1.5)}$$

Setup for forming the Laplace transform of $y^{[1]}$, $\mathcal{L}(y^{[1]})$, and checking if it is in the canonical form.

To derive $\mathcal{L}(y^{[1]}) = C_1 (sI - A_1)^{-1} x^{[1]}_0$, start by forming the $(sI - A_1)$ matrix

```
> prelim1:=MatrixAdd(IdentityMatrix(3,3),A1,s,-1);
```

(E3.2.1)

$$prelim1 := \begin{bmatrix} \alpha_1 k_a + s & -k_d & 0 \\ -k_a \alpha_1 & s + k_d + k_2 & -k_{-2} \\ 0 & -k_2 & s + k_{-2} \end{bmatrix} \quad \textbf{(E3.2.1)}$$

from which $\mathcal{L}(\mathbf{x}^{[1]})$ is given by

```
> LT_x1:=MatrixMatrixMultiply(MatrixInverse(prelim1),x0_1);
```

$LT\_x1 :=$ **(E3.2.2)**

$$\begin{bmatrix} \frac{\left(s^2 + s k_{-2} + s k_2 + s k_d + k_{-2} k_d\right) \beta_1}{s \left(s \alpha_1 k_a + \alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + s^2 + s k_{-2} + s k_2 + s k_d + k_{-2} k_d\right)} \\ \frac{k_a \alpha_1 \left(s + k_{-2}\right) \beta_1}{s \left(s \alpha_1 k_a + \alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + s^2 + s k_{-2} + s k_2 + s k_d + k_{-2} k_d\right)} \\ \frac{k_a \alpha_1 k_2 \beta_1}{s \left(s \alpha_1 k_a + \alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + s^2 + s k_{-2} + s k_2 + s k_d + k_{-2} k_d\right)} \end{bmatrix}$$

Collecting terms gives

```
> LT_x1_simp:=map(rat_fn_simplify,LT_x1,sortlist);
```

$LT\_x1\_simp :=$ **(E3.2.3)**

$$\begin{bmatrix} \frac{\beta_1 s^2 + \left(k_{-2} + k_2 + k_d\right) \beta_1 s + k_d k_{-2} \beta_1}{s^3 + \left(\alpha_1 k_a + k_{-2} + k_2 + k_d\right) s^2 + \left(\left(k_{-2} + k_2\right) k_a \alpha_1 + k_d k_{-2}\right) s} \\ \frac{s \alpha_1 \beta_1 k_a + \alpha_1 \beta_1 k_{-2} k_a}{s^3 + \left(\alpha_1 k_a + k_{-2} + k_2 + k_d\right) s^2 + \left(\left(k_{-2} + k_2\right) k_a \alpha_1 + k_d k_{-2}\right) s} \\ \frac{k_a \alpha_1 k_2 \beta_1}{s^3 + \left(\alpha_1 k_a + k_{-2} + k_2 + k_d\right) s^2 + \left(\left(k_{-2} + k_2\right) k_a \alpha_1 + k_d k_{-2}\right) s} \end{bmatrix}$$

Here any common factors in the rational functions above are cancelled

```
> LT_x1_simp2:=map(rat_fn_simplify,map(normal,LT_x1_simp),
  sortlist);
```

$LT\_x1\_simp2 :=$ **(E3.2.4)**

$$\begin{bmatrix} \dfrac{\beta_1 s^2 + (k_{-2} + k_2 + k_d)\,\beta_1 s + k_d k_{-2} \beta_1}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d)\, s^2 + ((k_{-2} + k_2)\, k_a \alpha_1 + k_d k_{-2})\, s} \\ \dfrac{s \alpha_1 \beta_1 k_a + \alpha_1 \beta_1 k_{-2} k_a}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d)\, s^2 + ((k_{-2} + k_2)\, k_a \alpha_1 + k_d k_{-2})\, s} \\ \dfrac{k_a \alpha_1 k_2 \beta_1}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d)\, s^2 + ((k_{-2} + k_2)\, k_a \alpha_1 + k_d k_{-2})\, s} \end{bmatrix}$$

Let us check on pole-zero cancellation in **(E3.2.4)** by direct substitution. Cancellation clearly cannot occur in the third component.
The second component is checked for cancellation by substitution of its numerator zero into its denominator.

```
> simplify(subs(s=-k[-2],denom(LT_x1_simp2[2,1])) );
```

$$-k_{-2} k_2 (\alpha_1 k_a - k_{-2}) \qquad \textbf{(E3.2.5)}$$

which is zero when $k_{-2} = k_a \alpha_1$. While it is possible for **(E3.2.5)** to be satisfied for one particular injected analyte concentration α_1, the condition will not always be satisfied in a general experimental series where a range of values for α_1 is used.

The equilibrium state (state as $t \to \infty$) for this closed system $\mathcal{C}(\boldsymbol{\theta})$ is determined from $\mathcal{L}(\boldsymbol{x}^{[1]})$ by determining the limit as $s \to 0^+$.

```
> equilibrium_x1:=map(simplify,map(limit,LT_x1_simp2,s=0,
  right));
```

equilibrium_x1 := **(E3.2.6)**

$$\begin{bmatrix} \dfrac{\mathrm{signum}(k_d)\,\mathrm{signum}(k_{-2})\,\mathrm{signum}(\beta_1)\,\infty}{\mathrm{signum}(\alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + k_{-2} k_d)} \\ \dfrac{\mathrm{signum}(k_a)\,\mathrm{signum}(\alpha_1)\,\mathrm{signum}(k_{-2})\,\mathrm{signum}(\beta_1)\,\infty}{\mathrm{signum}(\alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + k_{-2} k_d)} \\ \dfrac{\mathrm{signum}(k_a)\,\mathrm{signum}(\alpha_1)\,\mathrm{signum}(k_2)\,\mathrm{signum}(\beta_1)\,\infty}{\mathrm{signum}(\alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + k_{-2} k_d)} \end{bmatrix}$$

Proceeding with the derivation of the Laplace transform of $y^{[1]}$,

```
> LT_y1:=MatrixMatrixMultiply(C1,LT_x1):
> LT_y1_simp:=rat_fn_simplify(simplify(LT_y1[1,1]),sortlist);
```

LT_y1_simp := **(E3.2.7)**

$$\frac{k_a \alpha_1 \beta_1 s + k_a \alpha_1 \beta_1 (k_{-2} + k_2)}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d) s^2 + ((k_{-2} + k_2) k_a \alpha_1 + k_d k_{-2}) s}$$

Given the Laplace transform of an output function as in **(E3.2.7)**, it is necessary to put the expression into its canonical form. This is achieved by cancelling any factors common to the numerator and denominator and ensuring that the denominator is monic.

Substitution of the zero of the numerator of **(E3.2.7)** into its denominator serves as check on whether the corresponding factor is common to both polynomials.

```
> test:=simplify(subs(s=-(k[-2]+k[2]),denom(LT_y1_simp)));
```

$$test := (k_{-2} + k_2) k_2 k_d \qquad \textbf{(E3.2.8)}$$

As all parameters are positive, **(E3.2.8)** cannot be zero which shows that pole-zero cancellation does not occur in $\mathcal{L}(y^{[1]})$ shown in **(E3.2.7)**.
Hence **(E3.2.7)** is in the canonical form and its coefficients of powers of s are moment invariants.

The denominator of **(E3.2.7)** is the product of s and a quadratic in s. Let us record the descriminant of this quadratic.

```
> y1_denom_coeffs:=PolynomialTools[CoefficientList](denom
  (LT_y1_simp), s, termorder=reverse);
   y1_descriminant:=sort(simplify(y1_denom_coeffs[2]^2 - 4*
  y1_denom_coeffs[1]*y1_denom_coeffs[3]),sortlist);
```

$$y1\_denom\_coeffs := [1, \alpha_1 k_a + k_{-2} + k_2 + k_d, \alpha_1 k_{-2} k_a + \alpha_1 k_2 k_a + k_{-2} k_d, 0]$$

$$y1\_descriminant := \alpha_1^2 k_a^2 + 2 \alpha_1 k_a k_d - 2 \alpha_1 k_a k_2 - 2 \alpha_1 k_a k_{-2} + k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2 \qquad \textbf{(E3.2.9)}$$

A useful expression for $x^{[1]}$

For i=1,2,3, the i-th eigenvalue of A_1 appearing in the column vector given below **(Note: the ordering of e-vals can change from run to run)**

```
> print("eigenvalues of A1"); interface(prettyprint = 2) : A1_evects
      := Eigenvectors(A1) : print(A1_evects[1]); interface(prettyprint
      = 3) :
```

"eigenvalues of A1"

$$\begin{bmatrix} 0 \\ -\frac{1}{2} k_a \alpha_1 - \frac{1}{2} k_{-2} - \frac{1}{2} k_2 - \frac{1}{2} k_d + \frac{1}{2} \sqrt{\%1} \\ -\frac{1}{2} k_a \alpha_1 - \frac{1}{2} k_{-2} - \frac{1}{2} k_2 - \frac{1}{2} k_d - \frac{1}{2} \sqrt{\%1} \end{bmatrix}$$

$$\%1 = \alpha_1^2 k_a^2 + 2 \alpha_1 k_a k_d - 2 \alpha_1 k_a k_2 - 2 \alpha_1 k_a k_{-2} + k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2$$

corresponds to the eigenvector given in column i of the 3x3 matrix below:

```
> interface(prettyprint=2): print("eigenvectors of A1");
      print(A1_evects[2]); # interface(prettyprint=3):
                    "eigenvectors of A1"
```

$$\begin{bmatrix} \frac{k_d k_{-2}}{k_2 k_a \alpha_1} & -\frac{k_d(\alpha_1 k_a - k_{-2})}{(\%2)\left(\frac{1}{2}k_a\alpha_1 - \frac{1}{2}k_{-2} - \frac{1}{2}k_2 - \frac{1}{2}k_d + \frac{1}{2}\sqrt{\%1}\right)} & -\frac{k_d(\alpha_1 k_a - k_{-2})}{(\%3)\left(\frac{1}{2}k_a\alpha_1 - \frac{1}{2}k_{-2} - \frac{1}{2}k_2 - \frac{1}{2}k_d - \frac{1}{2}\sqrt{\%} \right.} \\ \frac{k_{-2}}{k_2} & -\frac{\alpha_1 k_a - k_{-2}}{\%2} & -\frac{\alpha_1 k_a - k_{-2}}{\%3} \\ 1 & 1 & 1 \end{bmatrix}$$

$$\%1 = \alpha_1^2 k_a^2 + 2\alpha_1 k_a k_d - 2\alpha_1 k_a k_2 - 2\alpha_1 k_a k_{-2} + k_d^2 + 2k_d k_2 - 2k_d k_{-2} + k_2^2 + 2k_2 k_{-2} + k$$

$$\%2 = \frac{1}{2}k_a\alpha_1 - \frac{1}{2}k_{-2} + \frac{1}{2}k_2 + \frac{1}{2}k_d + \frac{1}{2}\sqrt{\%1}$$

$$\%3 = \frac{1}{2}k_a\alpha_1 - \frac{1}{2}k_{-2} + \frac{1}{2}k_2 + \frac{1}{2}k_d - \frac{1}{2}\sqrt{\%1}$$

The following sets up the matrices that allow the writing of $\boldsymbol{x}^{[1]}$ in the sum-of-exponentials form $\boldsymbol{x}^{[1]}(t) = \sum_{i=1}^{3} \boldsymbol{v}_i e^{\lambda_i t}$ $(0 = \lambda_1 > \lambda_2 > \lambda_3)$ that will be useful later.

```
> S := A1_evects[2]: Sinv := MatrixInverse(S):
```

This begins the derivation of the vector coefficient of exp(0) in terms of the matrices above. Using the eigenvector associated with the 0 eigenvalue allows the calculation of $\left(\boldsymbol{s}^{(1)'}\boldsymbol{x}_0\right)$. For $i = 1, 2, 3$, $\left(\boldsymbol{s}^{(i)'}\boldsymbol{x}_0\right)$ are shown below.

```
> interface(prettyprint=2): part1:=expand(simplify
  (MatrixMatrixMultiply(Sinv,x0_1))):  print(part1[1]);
  interface(prettyprint=3): print(part1[2]); print(part1[3])
  ;
```

$$\begin{bmatrix} \frac{1}{4}\frac{k_2 k_a \alpha_1 \left(-k_a\alpha_1 + \sqrt{\%1} + k_{-2} + k_2 + k_d\right)\left(k_a\alpha_1 + \sqrt{\%1} - k_{-2} - k_2 - k_d\right)\beta_1}{\left(\alpha_1^2 k_{-2} k_a^2 + \alpha_1^2 k_2 k_a^2 - \alpha_1 k_{-2}^2 k_a - \alpha_1 k_{-2} k_2 k_a + \alpha_1 k_{-2} k_a k_d - k_{-2}^2 k_d\right) k_d} \end{bmatrix}$$

$$\%1 = \alpha_1^2 k_a^2 + 2\alpha_1 k_a k_d - 2\alpha_1 k_a k_2 - 2\alpha_1 k_a k_{-2} + k_d^2 + 2k_d k_2 - 2k_d k_{-2} + k_2^2 + 2k_2 k_{-2} + k^2$$

$$\left[\frac{1}{16}\left(\alpha_1 k_a\left(-k_a\alpha_1 \right.\right.\right.$$

$$+\left(\alpha_1^2 k_a^2 + 2\alpha_1 k_a k_d - 2\alpha_1 k_a k_2 - 2\alpha_1 k_a k_{-2} + k_d^2 + 2k_d k_2\right.$$

$$\left.\left.\left.-2k_d k_{-2} + k_2^2 + 2k_2 k_{-2} + k_{-2}^2\right)^{1/2} + k_{-2} + k_2 + k_d\right)\left(k_a\alpha_1\right.\right.$$

$$+\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$\left.-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+k_{-2}^2\right)^{1/2}-k_{-2}-k_2-k_d\Big)\Big(k_a\alpha_1$$

$$+\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$\left.-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+k_{-2}^2\right)^{1/2}-k_{-2}+k_2+k_d\Big)\Big(-\alpha_1 k_a k_{-2}$$

$$-2\,\alpha_1 k_a k_2$$

$$+k_{-2}\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$\left.-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+k_{-2}^2\right)^{1/2}+k_{-2}^2+k_2 k_{-2}-k_d k_{-2}\Big)\beta_1\Big)\Big/\Big(($$

$$\alpha_1^2 k_{-2} k_a^2+\alpha_1^2 k_2 k_a^2-\alpha_1 k_{-2}^2 k_a-\alpha_1 k_{-2} k_2 k_a+\alpha_1 k_{-2} k_a k_d-k_{-2}^2 k_d\Big)$$

$$\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+\right.$$

$$\left.k_{-2}^2\right)^{1/2}(\alpha_1 k_a-k_{-2})\,k_d\Big)\Big]$$

$$\Big[-\frac{1}{16}\Big(\alpha_1 k_a\Big(-k_a\alpha_1 \qquad \textbf{(E3.2.}$$

$$+\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$\left.-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+k_{-2}^2\right)^{1/2}+k_{-2}+k_2+k_d\Big)\Big(-k_a\alpha_1$$

$$+\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$\left.-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+k_{-2}^2\right)^{1/2}+k_{-2}-k_2-k_d\Big)\Big(k_a\alpha_1$$

$$+\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$\left.-2\,k_d k_{-2}+k_2^2+2\,k_2 k_{-2}+k_{-2}^2\right)^{1/2}-k_{-2}-k_2-k_d\Big)\Big(\alpha_1 k_a k_{-2}$$

$$+2\,\alpha_1 k_a k_2$$

$$+k_{-2}\left(\alpha_1^2 k_a^2+2\,\alpha_1 k_a k_d-2\,\alpha_1 k_a k_2-2\,\alpha_1 k_a k_{-2}+k_d^2+2\,k_d k_2\right.$$

$$-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2\Big)^{1/2}-k_{-2}^2-k_2\,k_{-2}+k_d\,k_{-2}\Big)\,\beta_1\Big)\Big/\Big(\Big($$

$$\alpha_1^2\,k_{-2}\,k_a^2+\alpha_1^2\,k_2\,k_a^2-\alpha_1\,k_{-2}^2\,k_a-\alpha_1\,k_{-2}\,k_2\,k_a+\alpha_1\,k_{-2}\,k_a\,k_d-k_{-2}^2\,k_d\Big)$$

$$\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+$$

$$k_{-2}^2\Big)^{1/2}\,(\alpha_1\,k_a-k_{-2})\,k_d\Big)\Big]$$

For i = 1, $\left(s^{(i)'}\,x_0\right)$ multiplies the column vector s_i to give the equilibrium state of $x^{[1]}$:

```
> interface(prettyprint=3): zero_eval_coeff_vec:= map
  (simplify,map(expand,ScalarMultiply(Column(S,1),part1[1]))
  ); interface(prettyprint=3):
```

$$zero\_eval\_coeff\_vec:=\begin{bmatrix}\dfrac{k_d\,k_{-2}\,\beta_1}{\alpha_1\,k_{-2}\,k_a+\alpha_1\,k_2\,k_a+k_{-2}\,k_d}\\[2ex]\dfrac{k_a\,\alpha_1\,k_{-2}\,\beta_1}{\alpha_1\,k_{-2}\,k_a+\alpha_1\,k_2\,k_a+k_{-2}\,k_d}\\[2ex]\dfrac{k_a\,\alpha_1\,k_2\,\beta_1}{\alpha_1\,k_{-2}\,k_a+\alpha_1\,k_2\,k_a+k_{-2}\,k_d}\end{bmatrix}\qquad \textbf{(E3.2.1.4)}$$

as seen in **(E3.2.6)**. The other vectors to appear in the expression for $x^{[1]}$ are calculated similarly. Starting from the term corresponding to the largest of the negative eigenvalues, λ_2:

```
> lambda2_eval_coeff_vec := map(rat_fn_simplify, map(expand,
  ScalarMultiply(Column(S, 2), part1[2])),sortlist):
> radicand_list:=[y1_descriminant]:
> lambda2_coeff_vec:=map(collect_radicals,
  lambda2_eval_coeff_vec, radicand_list, sortlist);
```

$$lambda2\_coeff\_vec:=\Big[\Big[\Big(\Big(2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2 \qquad \textbf{(E3.2.}$$

$$+2\,k_d\,k_2-2\,k_d\,k_{-2}$$

$$+\left(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2\right.$$

$$\left.-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2\right)^{1/2}\,k_d+(2\,k_{-2}+2\,k_2-k_d)\,k_a\,\alpha_1$$

$$+(3\,k_{-2}-k_2)\,k_d\Big)\,\alpha_1\,\beta_1\,k_a\,k_2\Big)\Big/\Big(\Big(\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2$$

$$
\begin{aligned}
&-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2\Big)\,k_{-2}\\
&+\big((k_{-2}+k_2)\,k_a\,\alpha_1\\
&+k_d\,k_{-2}\big)\\
&\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+\\
&k_{-2}^2\Big)^{1/2}+\alpha_1^2\,k_a^2\,k_2+\big(k_{-2}^2-k_2^2+k_2\,k_d\big)\,k_a\,\alpha_1+\big(k_{-2}^2-3\,k_{-2}\,k_2\big)\,k_d-\\
&k_{-2}^3-2\,k_2\,k_{-2}^2-k_2^2\,k_{-2}\Big)\\
&\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+\\
&k_{-2}^2\Big)^{1/2}\Big)\Big],\\
&\Big[\Big(\Big(\big(\alpha_1\,k_a\\
&-k_{-2}\big)\\
&\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2\\
&+2\,k_2\,k_{-2}+k_{-2}^2\Big)^{1/2}+\alpha_1^2\,k_a^2+(k_2+k_d)\,k_a\,\alpha_1-k_{-2}^2-k_2\,k_{-2}\\
&-k_d\,k_{-2}\Big)\,\alpha_1\,\beta_1\,k_a\,k_2\Big)\Big/\Big(\Big(\big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2\\
&-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2\big)\,k_{-2}\\
&+\big((k_{-2}+k_2)\,k_a\,\alpha_1\\
&+k_d\,k_{-2}\big)\\
&\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+\\
&k_{-2}^2\Big)^{1/2}+\alpha_1^2\,k_a^2\,k_2+\big(k_{-2}^2+2\,k_{-2}\,k_2+k_2^2+k_2\,k_d\big)\,k_a\,\alpha_1+\big(k_{-2}^2\\
&-k_{-2}\,k_2\big)\,k_d-k_{-2}^3-2\,k_2\,k_{-2}^2-k_2^2\,k_{-2}\Big)\\
&\Big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+
\end{aligned}
$$

$$k_{-2}^2\Big)^{1/2}\Big)\Big],$$

$$\Big[-\frac{1}{2}\,\frac{k_a\,\alpha_1\,k_2\,\beta_1}{(k_{-2}+k_2)\,k_a\,\alpha_1+k_d\,k_{-2}}-\frac{1}{2}\,\big((\alpha_1\,k_a+k_{-2}+k_2$$

$$+k_d)\,\alpha_1\,\beta_1\,k_a\,k_2\big)\Big/\Big(\big((k_{-2}+k_2)\,k_a\,\alpha_1$$

$$+k_d\,k_{-2}\big)$$

$$\big(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+$$

$$k_{-2}^2\Big)^{1/2}\Big)\Big]\Big]$$

Collection of moment invariants $\phi^{[1]}$ from $\mathcal{C}^{[1]}$ for later use in an identifiability test.

```
> phi[1] := coeff(numer(LT_y1_simp), s, 1);
```

$$\phi_1 := k_a\,\alpha_1\,\beta_1 \qquad \textbf{(E3.3.1)}$$

```
> phi[2] := coeff(numer(LT_y1_simp), s, 0);
```

$$\phi_2 := k_a\,\alpha_1\,\beta_1\,(k_{-2}+k_2) \qquad \textbf{(E3.3.2)}$$

```
> phi[3] := collect(coeff(denom(LT_y1_simp), s, 2),sortlist);
```

$$\phi_3 := \alpha_1\,k_a+k_{-2}+k_2+k_d \qquad \textbf{(E3.3.3)}$$

```
> phi[4] := collect(coeff(denom(LT_y1_simp), s, 1),sortlist);
```

$$\phi_4 := (k_{-2}+k_2)\,k_a\,\alpha_1+k_d\,k_{-2} \qquad \textbf{(E3.3.4)}$$

As there are five parameters and only four invariants available from $\mathcal{C}^{[1]}$, it is not possible to show that $\mathcal{C}$ is globally *a priori* identifiable using only the information obtainable from a typical association phase of an experiment. Hence, it is necessary to proceed to consider the information obtainable from the dissociation phase.

Vector of alternative parameter values:

```
> theta_prime:=[Kappa[a],Kappa[d],Kappa[2],Kappa[-2],B[1]];
```

$$theta\_prime := [\mathrm{K}_a, \mathrm{K}_d, \mathrm{K}_2, \mathrm{K}_{-2}, B_1] \qquad \textbf{(E3.3.5)}$$

Using the invariants above to set up the test equations:

```
> for i from 1 to 4 do: eq[i]:=phi[i]=subs({k[a]=Kappa[a],k[d]
  =Kappa[d],beta[1]=B[1],k[2]=Kappa[2],k[-2]=Kappa[-2]},phi[i]
  );  od;
```

$$eq_1 := k_a\,\alpha_1\,\beta_1 = \mathrm{K}_a\,\alpha_1\,B_1$$

$$eq_2 := k_a \alpha_1 \beta_1 \left(k_{-2} + k_2\right) = \mathrm{K}_a \alpha_1 B_1 \left(\mathrm{K}_{-2} + \mathrm{K}_2\right)$$

$$eq_3 := \alpha_1 k_a + k_{-2} + k_2 + k_d = \mathrm{K}_a \alpha_1 + \mathrm{K}_{-2} + \mathrm{K}_2 + \mathrm{K}_d$$

$$eq_4 := \left(k_{-2} + k_2\right) k_a \alpha_1 + k_d k_{-2} = \left(\mathrm{K}_{-2} + \mathrm{K}_2\right) \mathrm{K}_a \alpha_1 + \mathrm{K}_d \mathrm{K}_{-2} \qquad \textbf{(E3.3.6)}$$

```
> IDequations_assoc_phase := []; for i to 4 do
  IDequations_assoc_phase := [op(IDequations_assoc_phase), eq
  [i]] end do:
```

$$IDequations\_assoc\_phase := [\,] \qquad \textbf{(E3.3.7)}$$

and solving gives

```
> solset1:=solve(IDequations_assoc_phase,theta_prime);
```

$$solset1 := \left[\left[\mathrm{K}_a = \frac{\mathrm{K}_{-2} \alpha_1 k_a - \alpha_1 k_{-2} k_a - \alpha_1 k_2 k_a + \mathrm{K}_{-2} k_d - k_{-2} k_d}{\alpha_1 \left(\mathrm{K}_{-2} - k_2 - k_{-2}\right)}, \mathrm{K}_d = -\frac{k_d k_2}{\mathrm{K}_{-2} - k_2 - k_{-2}}, \mathrm{K}_2 = -\mathrm{K}_{-2} + k_2 + k_{-2}, \mathrm{K}_{-2} = \mathrm{K}_{-2}, B_1 = \frac{k_a \beta_1 \alpha_1 \left(\mathrm{K}_{-2} - k_2 - k_{-2}\right)}{\mathrm{K}_{-2} \alpha_1 k_a - \alpha_1 k_{-2} k_a - \alpha_1 k_2 k_a + \mathrm{K}_{-2} k_d - k_{-2} k_d}\right]\right] \qquad \textbf{(E3.3.8)}$$

4 Consideration of the structure $\mathcal{C}^{[2]}$

Setting up a general system in $\mathcal{C}^{[2]}$

For this Maple worksheet, initial conditions $\boldsymbol{x}^{[2]}_0 = \left(x^{[2]}_{0,1}, x^{[2]}_{0,2}, x^{[2]}_{0,3}\right)$ are specified as

```
> x0_2:=Matrix(3,1,[x0[2,1],x0[2,2],x0[2,3]]);
```

$$x0\_2 := \begin{bmatrix} x0_{2,1} \\ x0_{2,2} \\ x0_{2,3} \end{bmatrix} \qquad \textbf{(E4.1.1)}$$

which ignores the conservation of mass constraint (applied here to the phase's initial state) $x^{[2]}_{0,1} + x^{[2]}_{0,2} + x^{[2]}_{0,3} = \beta_1$ in the interest of algebraic simplicity. We will consider this condition later.

We assume that $x^{[2]}_{0,1}$, $x^{[2]}_{0,2}$, $x^{[2]}_{0,3} > 0$ as if the association phase is allowed to occur for even a very short time, mass would be distributed between the three states by the start of the dissociation phase of an experiment.

The matrix A_2 (representing the dynamics of the dissociation phase) is obtained

from A_1 by setting analyte concentration α_1 to zero.

```
> A2:=Matrix(3,3,[]):
> for i to 3 do for j to 3 do A2[i, j] := subs(alpha[1] = 0,
  A1[i, j]) end do end do; print(A2);
```

$$\begin{bmatrix} 0 & k_d & 0 \\ 0 & -k_d - k_2 & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix} \quad \textbf{(E4.1.2)}$$

The same observation gain matrix operates in both phases of an experiment, hence **C2**=**C1**

```
> C2 := Matrix(C1);
```

$$C2 := \begin{bmatrix} 0 & 1 & 1 \end{bmatrix} \quad \textbf{(E4.1.3)}$$

Setting up the Laplace transform of $y^{[2]}$, $\mathcal{L}(y^{[2]}) = C_2 \left(sI - A_2\right)^{-1} x_0^{[2]}$

Starting from $\left(sI - A_2\right)$:

```
> prelim2 := MatrixAdd(IdentityMatrix(3,3), A2, s,-1);
```

$$prelim2 := \begin{bmatrix} s & -k_d & 0 \\ 0 & s + k_d + k_2 & -k_{-2} \\ 0 & -k_2 & s + k_{-2} \end{bmatrix} \quad \textbf{(E4.2.1)}$$

```
> LT_x2:=MatrixMatrixMultiply(MatrixInverse(prelim2),x0_2):
> LT_y2:=simplify(MatrixMatrixMultiply(C2,LT_x2)[1,1]);
```

$LT\_y2 :=$ **(E4.2.2)**

$$\frac{s\,x0_{2,2} + s\,x0_{2,3} + k_{-2}\,x0_{2,2} + k_{-2}\,x0_{2,3} + k_2\,x0_{2,2} + k_2\,x0_{2,3} + k_d\,x0_{2,3}}{s^2 + s\,k_{-2} + s\,k_2 + s\,k_d + k_{-2}\,k_d}$$

```
> LT_y2_simp:=collect(numer(LT_y2),[s,x0[2,2],x0[2,3]])
  /collect(denom(LT_y2),s);
```

$$LT\_y2\_simp := \frac{(x0_{2,2} + x0_{2,3})\,s + (k_{-2} + k_2)\,x0_{2,2} + (k_{-2} + k_2 + k_d)\,x0_{2,3}}{s^2 + (k_{-2} + k_2 + k_d)\,s + k_d\,k_{-2}} \quad \textbf{(E4.2.3)}$$

At this point it is not known whether $\mathcal{L}(y^{[2]})$ given by **(E4.2.3)** is in the required canonical form. Features of the denominator of **(E4.2.3)** will be useful in addressing this later.

```
> y2_LT_denom_coeff:=  PolynomialTools[CoefficientList](denom
  (LT_y2_simp), s, termorder=reverse);
```

$$y2\_LT\_denom\_coeff := \left[1,\ k_{-2} + k_2 + k_d,\ k_d\,k_{-2}\right] \quad \textbf{(E4.2.4)}$$

```
> y2_descrim:=sort(expand(y2_LT_denom_coeff[2]^2 - 4*
```

```
y2_LT_denom_coeff[1]*y2_LT_denom_coeff[3]),sortlist);
```

$$y2\_descrim := k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2 \qquad \textbf{(E4.2.5)}$$

5 Investigation of factor cancellation in $\mathcal{L}\left(y^{[2]}\right)$: an approach with detail on initial conditions

Consider the currently unprocessed form of $\mathcal{L}\left(y^{[2]}\right)$, **(E4.2.3)** .

To determine the information obtainable from $\mathcal{C}^{[2]}$ it is necessary to determine the canonical form of **(E4.2.3)**. This is approached by considering the possibility of cancellation of factors in the rational function.

The following approach is more fully described in Chapter 3.

If substitution of a zero of the denominator of **(E4.2.3)** into its numerator gives zero, then it is necessary to cancel the associated linear factor from the numerator and denominator. Repeating this process for all zeroes of the denominator and ensuring that the denominator is monic will put **(E4.2.3)** into the canonical form. A subset of the total set of invariants may then be obtained which excludes any numerator terms which depend on $x_0^{[2]}$, the initial conditions of a general system in structure $\mathcal{C}^{[2]}$. By combining this subset with $\phi^{[1]}$, a test of $\mathcal{C}$ for global a priori identifiability can be applied. The actual classification of the model structure cannot be worse than the result obtained by this process. Hence, the process is sufficient to demonstrate that a structure is globally *a priori* identifiable.

Determine the zeroes of the denominator of **(E4.2.3)**.

```
> denom_zeroes:=[solve(denom(LT_y2),s)]:
```

Let us denote the larger of these two zeroes by λ_l and the smaller by λ_s.

```
> lambda[l]:=denom_zeroes[1];
```

$$\lambda_l := -\frac{1}{2}\,k_{-2} - \frac{1}{2}\,k_2 - \frac{1}{2}\,k_d + \frac{1}{2}\,\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} \qquad \textbf{(E5.1)}$$

```
> lambda[s] := denom_zeroes[2];
```

$$\lambda_s := -\frac{1}{2}\,k_{-2} - \frac{1}{2}\,k_2 - \frac{1}{2}\,k_d - \frac{1}{2}\,\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} \qquad \textbf{(E5.2)}$$

Check for cancellation of $\left(s - \lambda_l\right)$ in (E4.2.3).

Substitute λ_l back into the denominator to confirm that it is a zero.

```
> check1:=simplify(subs(s=lambda[l],denom(LT_y2)));
```

$$check1 := 0 \qquad \textbf{(E5.1.1)}$$

Having passed the check, substituting λ_l into the numerator of **(E4.2.3)** and

equating to zero gives a condition on when λ_l is a zero of the polynomial.

```
> cancellation_condition:=collect(simplify(subs(s=lambda[l],
  numer(LT_y2))),[x0[2,2],x0[2,3]])=0;
```

$$cancellation\_condition := \left(\frac{1}{2} k_{-2} + \frac{1}{2} k_2 - \frac{1}{2} k_d + \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \right) x0_{2,2} + \left(\frac{1}{2} k_{-2} + \frac{1}{2} k_2 + \frac{1}{2} k_d + \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \right) x0_{2,3} = 0 \quad \textbf{(E5.1.2)}$$

First, let us see if we can make an argument on when **(E5.1.2)** is satisfied, and hence when cancellation of $(s - \lambda_l)$ occurs in **(E4.2.3)**, without requiring explicit expressions for the initial conditions of $\mathcal{C}^{[2]}$. Note that the coefficient of $x_{0,3}^{[2]}$ in **(E5.1.2)** is strictly real and positive for this physical compartmental system with positive parameters. (As the system is catenary in type, the expression under the radical is strictly positive.) Any solution to **(E5.1.2)** requires that the coefficient of $x_{0,2}^{[2]}$ is negative.

If the solutions of **(E5.1.2)** are all unphysical, it is reasonable to rule out cancellation of the linear term under consideration.

Note the following constraints on the radical term in **(E5.1.2)**.

```
> lower_constraint:= abs(k[2] + k[-2] - k[d]) < (sort(k[2]^2+2*
  k[-2]*k[2]-2*k[d]*k[-2]+2*k[d]*k[2]+k[-2]^2 +k[d]^2,
  sortlist))^(1/2) ;
```

$$lower\_constraint := |k_{-2} + k_2 - k_d| < \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \quad \textbf{(E5.1.3)}$$

```
> upper_constraint:= ( k[2]^2+2*k[-2]*k[2]-2*k[d]*k[-2]+2*k[d]
  *k[2]+k[-2]^2 +k[d]^2)^(1/2) < k[2] + k[-2] + k[d] ;
```

$$upper\_constraint := \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} < k_{-2} + k_2 + k_d \quad \textbf{(E5.1.4)}$$

which is expected as a consequence of λ_l<0 for all feasible **θ**. To demonstrate that **(E5.1.4)** holds, as both sides of the expression are positive, squaring doesn't change the sign of the inequality. Hence

```
> simplify(rhs(upper_constraint)^2 - lhs(upper_constraint)^2
  >0);
```

$$0 < 4 k_d k_{-2} \quad \textbf{(E5.1.5)}$$

which is satisfied for all feasible parameter values.

Under that assumption that the coefficient of $x_{0,2}^{[2]}$ must be negative in **(E5.1.2)**,

in order for solutions to exist:

```
> k[2]+k[-2]-k[d]+sqrt(k[2]^2+2*k[-2]*k[2]-2*k[d]*k[-2]+2*k[d]
  *k[2]+k[-2]^2+k[d]^2) <0 ;
```

$$k_{-2} + k_2 - k_d + \sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} < 0 \qquad \textbf{(E5.1.6)}$$

rearranging gives

```
> cancellation_condition:=sqrt(k[2]^2+2*k[-2]*k[2]+2*k[2]*k[d]
  +k[-2]^2-2*k[d]*k[-2]+k[d]^2)< -k[2]-k[-2]+k[d];
```

$$cancellation\_condition := \sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} < -k_2 - k_{-2} + k_d \qquad \textbf{(E5.1.7)}$$

As the left-hand side of **(E5.1.7)** is positive, the right-hand side is also. Thus, squaring both sides does not change the direction of the inequality, giving

```
> cancellation_condition:=lhs(cancellation_condition)^2 < rhs
  (cancellation_condition)^2 ;
```

$$cancellation\_condition := k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2 < (-k_2 - k_{-2} + k_d)^2 \qquad \textbf{(E5.1.8)}$$

Rearranging

```
> cancellation_condition:= 0 < rhs((E5.1.8)) - lhs((E5.1.8))
```

$$cancellation\_condition := 0 < (-k_2 - k_{-2} + k_d)^2 - k_{-2}^2 - 2\,k_2\,k_{-2} + 2\,k_d\,k_{-2} - k_2^2 - 2\,k_d\,k_2 - k_d^2 \qquad \textbf{(E5.1.9)}$$

and simplifying we obtain

```
> cancellation_condition:=simplify(cancellation_condition);
```

$$cancellation\_condition := 0 < -4\,k_d\,k_2 \qquad \textbf{(E5.1.10)}$$

As k_d and k_2 are both positive, **(E5.1.10)** is not satisfied anywhere. Hence, solutions to **(E5.1.2)** cannot give a negative coefficient of $x_{0,2}^{[2]}$ if λ_l from **(E5.1)** is a zero of the numerator of **(E4.2.3)** . We conclude that cancellation of the factor $(s - \lambda_l)$ in **(E4.2.3)** does not occur for any feasible parameter values.

A similar method is employed when considering the remaining zero of the denominator of **(E4.2.3)**, λ_s.

Check for cancellation of $(s - \lambda_s)$ in (E4.2.3)

To verify that λ_s (Equation **(E5.2)**) is a zero of the denominator of **(E4.2.3)** (that is, $\mathcal{L}(y^{[2]})$)

```
> check2:=simplify(subs(s=lambda[s],denom(LT_y2)));
```

$$check2 := 0 \qquad \textbf{(E5.2.1)}$$

Having shown this, let us look for conditions under which the numerator of **(E4.2.3)** with $s = \lambda_s$ is zero. Start by forming the equation

```
> can_con2:=collect(simplify(subs(s=lambda[s],numer(LT_y2))),
  [x0[2,2],x0[2,3]])=0;
```

$$can\_con2 := \left(\frac{1}{2} k_{-2} + \frac{1}{2} k_2 - \frac{1}{2} k_d - \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \right) x0_{2,2} + \left(\frac{1}{2} k_{-2} + \frac{1}{2} k_2 + \frac{1}{2} k_d - \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \right) x0_{2,3} = 0 \qquad \textbf{(E5.2.2)}$$

```
> xi2_coeff:=coeff(lhs(can_con2),x0[2,2]);
```

$$xi2\_coeff := \frac{1}{2} k_{-2} + \frac{1}{2} k_2 - \frac{1}{2} k_d - \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \qquad \textbf{(E5.2.3)}$$

```
> xi3_coeff:=coeff(lhs(can_con2),x0[2,3]);
```

$$xi3\_coeff := \frac{1}{2} k_{-2} + \frac{1}{2} k_2 + \frac{1}{2} k_d - \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \qquad \textbf{(E5.2.4)}$$

Recalling λ_l from **(E5.1)** (also one of the eigenvalues of the compartmental linear time-invariant system $\mathcal{C}^{[2]}$ and hence non-positive)

```
> lambda[l];
```

$$-\frac{1}{2} k_{-2} - \frac{1}{2} k_2 - \frac{1}{2} k_d + \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \qquad \textbf{(E5.2.5)}$$

it is seen that the coefficient of $x_{0,3}^{[2]}$ in **(E5.2.2)** is $-\lambda_l$ and hence it is positive. To satisfy **(E5.2.2)** the coefficient of $x_{0,2}^{[2]}$ must be negative:

```
> condition[2]:=xi2_coeff<0;
```

$$condition_2 := \frac{1}{2} k_{-2} + \frac{1}{2} k_2 - \frac{1}{2} k_d - \frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} < 0 \qquad \textbf{(E5.2.6)}$$

Rearranging gives

```
> condition[2]:= sqrt(k[2]^2+2*k[-2]*k[2]-2*k[d]*k[-2]+2*k[2]*
  k[d]+k[-2]^2+k[d]^2) > k[-2]+k[2]-k[d];
```

$$condition_2 := k_{-2} + k_2 - k_d < \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} \qquad \textbf{(E5.2.7)}$$

From **(E5.1.3)**, **(E5.2.7)** is always satisfied.

Hence, we cannot rule out cancellation of the $(s - \lambda_s)$ factor in **(E4.2.3)** using this approach.

6 A consideration of a cancellation condition of $\mathcal{L}(y^{[2]})$ holding on a time interval

Recall that the cancellation condition for λ_l is not satisfied for any feasible parameter values.
In order to claim that **(E4.2.3)** is generically minimal, we would need to prove an equivalent result for λ_s.

Let us consider when a condition such as $d_2\xi_2 + d_3\xi_3 = 0$ may hold on a subinterval of the time set $(0,+\infty)$.
Recall ξ_2, ξ_3 are sums of exponentials with exponents that are the eigenvalues of A_1. These are given by the denominator of **(E4.2.3)**, and the largest of these is 0, which is henceforth denoted by λ_1. The states $\boldsymbol{\xi}$ (equivalently $x^{[1]}$) may be written in terms of the matrix of eigenvectors S, and S^{-1}. As the exponentials are linearly independent (as seen by forming a Wronskian of the three terms), the equation $d_2\xi_2 + d_3\xi_3 = 0$ is satisfied when the coefficients of the exponentials are all zero simultaneously. The simplest of these three conditions arises from the coefficient of the constant term (e^0) as the eigenvector associated with λ_1 (that is $s_1 = (s_{11}, s_{21}, s_{31})'$) is of a simpler form than the other two eigenvectors.
The condition of interest is $\left(s^{(1)'} x_0\right)\left(d_2 s_{21} + d_3 s_{31}\right) = 0.$

For a closed, uncontrolled system as given by $\mathcal{C}^{[1]}(\boldsymbol{\theta})$, $\left(s^{(1)'} x_0\right)\mathbf{s}_1 = x^{[1]^*}$, which is the equilibrium state **(E3.2.1.4)**.
Hence, the condition reduces to $d_2 x_2^{[1]^*} + d_3 x_3^{[1]^*} = 0$. The left-hand side is the value of the cancellation condition as $t \to +\infty$.

```
> extended_cc_const_coeff1:= rationalize(xi2_coeff*
  zero_eval_coeff_vec[2,1] + xi3_coeff*zero_eval_coeff_vec[3,1],
  [y2_descrim],sortlist);
```

extended_cc_const_coeff1 := **(E6.1)**

$$-\frac{1}{2\,\alpha_1\,k_{-2}\,k_a + 2\,\alpha_1\,k_2\,k_a + 2\,k_{-2}\,k_d}\left(k_a\,\alpha_1\,\beta_1\left(k_{-2}\right.\right.$$

$$\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2}$$

$$\left. + k_2\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} - k_{-2}^2 - 2\,k_2\,k_{-2} + k_d\,k_{-2} - \right.$$

$$\left.\left. k_2^2 - k_d\,k_2\right)\right)$$

```
> const_coeff_zero_solns := solve(extended_cc_const_coeff1=0,
  theta);
```

$$const\_coeff\_zero\_solns := \left[\left[\beta_1 = 0, k_a = k_a, k_d = k_d, k_2 = k_2, k_{-2} = k_{-2}\right], \left[\beta_1 = \beta_1, k_a = 0, k_d = k_d, k_2 = k_2, k_{-2} = k_{-2}\right], \left[\beta_1 = \beta_1, k_a = k_a, k_d = 0, k_2 = k_2, k_{-2} = k_{-2}\right], \left[\beta_1 = \beta_1, k_a = k_a, k_d = k_d, k_2 = 0, k_{-2} = k_{-2}\right], \left[\beta_1 = \beta_1, k_a = k_a, k_d = k_d, k_2 = k_2, k_{-2} = 0\right]\right] \quad \textbf{(E6.2)}$$

Each of the solutions in **(E6.2)** are infeasible as they do not satisfy the requirement that all parameters are strictly positive. Thus, **(E6.1)** is not zero within the feasible parameter space. This shows that **(E5.2.2)** is not zero for all time.
This judgement is made without needing to consider the conditions which arise from requiring the coefficients of the other two exponential terms to be zero. However, we still have the problem of determining where the cancellation condition is satisfied.

While this has shown that $d_2\xi_2 + d_3\xi_3 = 0$ does not hold on a subinterval of $(0, +\infty)$. However, we cannot preclude the existence of isolated points for which the equality does hold.

An alternative approach using the Laplace transform of $x^{[1]}$

Let us consider an alternative approach to ascertaining the parameter values such that cancellation condition as in **(E5.2.2)** can hold.

The requirement that a cancellation condition associated with the factor $(s - \lambda_s)$ holds imposes a relationship between the elements of $\boldsymbol{x}^{[1]}$.
Let us suppose that this relationship holds for all time.
In such a case, The terms ξ_2 and ξ_3 in the cancellation condition are replaced by $x_2^{[1]}(t, \boldsymbol{\theta})$ and $x_3^{[1]}(t, \boldsymbol{\theta})$ respectively,
and $d_2\, x_2^{[1]}(t, \boldsymbol{\theta}) + d_3\, x_3^{[1]}(t, \boldsymbol{\theta}) = 0 \;\forall\; t$ in the closure of $\mathbb{R}_+$.
Rather than approach this directly, let us take the Laplace transform of the expression. Recall the Laplace transform of $\boldsymbol{x}^{[1]}$ given by **(E3.2.4)**:

```
> LT_x1_simp2;
```

(E6.1.1)

$$\begin{bmatrix} \dfrac{\beta_1 s^2 + (k_{-2} + k_2 + k_d)\,\beta_1 s + k_d k_{-2} \beta_1}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d)\, s^2 + ((k_{-2} + k_2)\, k_a \alpha_1 + k_d k_{-2})\, s} \\ \dfrac{s \alpha_1 \beta_1 k_a + \alpha_1 \beta_1 k_{-2} k_a}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d)\, s^2 + ((k_{-2} + k_2)\, k_a \alpha_1 + k_d k_{-2})\, s} \\ \dfrac{k_a \alpha_1 k_2 \beta_1}{s^3 + (\alpha_1 k_a + k_{-2} + k_2 + k_d)\, s^2 + ((k_{-2} + k_2)\, k_a \alpha_1 + k_d k_{-2})\, s} \end{bmatrix} \quad \textbf{(E6.1.1)}$$

and also recall from Section 3 that pole-zero cancellation does not occur in the second and third components of **(E6.1.1)**.
The condition of interest now relates the Laplace transform of the state variable vector by

```
> cond:= collect_radicals(xi2_coeff*numer(LT_x1_simp[2,1]) +
  xi3_coeff*numer(LT_x1_simp[3,1])=0 ,[y2_descrim],sortlist);
```

$$cond := \left(-\frac{1}{2} k_a \alpha_1 \beta_1 s + \left(-\frac{1}{2} k_{-2} - \frac{1}{2} k_2 \right) k_a \beta_1 \alpha_1 \right) \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} + \left(\frac{1}{2} k_{-2} + \frac{1}{2} k_2 - \frac{1}{2} k_d \right) k_a \beta_1 \alpha_1 s + \left(\left(-\frac{1}{2} k_{-2} + \frac{1}{2} k_2 \right) k_d + \frac{1}{2} k_{-2}^2 + k_2 k_{-2} + \frac{1}{2} k_2^2 \right) k_a \beta_1 \alpha_1 = 0 \quad \textbf{(E6.1.2)}$$

For equality to hold, the coefficients of s and the constant term on the left of **(E6.1.2)** must equal zero. The condition on the coefficient of s is

```
> rel[1]:=coeff(lhs(cond),s,1) = 0 ;
```

$$rel_1 := -\frac{1}{2} \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2}\, \alpha_1 \beta_1 k_a + \left(\frac{1}{2} k_{-2} + \frac{1}{2} k_2 - \frac{1}{2} k_d \right) k_a \beta_1 \alpha_1 = 0 \quad \textbf{(E6.1.3)}$$

and similarly, the condition on the constant term is

```
> rel[2]:=coeff(lhs(cond),s,0) = 0 ;
```

$$rel_2 := \left(-\frac{1}{2} k_{-2} - \frac{1}{2} k_2 \right) k_a \beta_1 \alpha_1 \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} + \left(\left(-\frac{1}{2} k_{-2} + \frac{1}{2} k_2 \right) k_d + \frac{1}{2} k_{-2}^2 + k_2 k_{-2} + \frac{1}{2} k_2^2 \right) k_a \beta_1 \alpha_1 = 0 \quad \textbf{(E6.1.4)}$$

Solving these relations for $\boldsymbol{\theta}$ gives

```
> theta_sol_set:=solve([rel[1],rel[2]],theta);
```

$$theta\_sol\_set := \left[\left[\beta_1 = \beta_1,\ k_a = 0,\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = 0,\ k_a = k_a,\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = 0,\ k_2 = k_2,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = k_d,\ k_2 = 0,\ k_{-2} = k_{-2}\right]\right] \quad \textbf{(E6.1.5)}$$

The solutions in **(E6.1.5)** are all implausible as parameters are strictly positive, not zero. From this we conclude that the parameters do not allow the cancellation condition associated with $(s - \lambda_s)$ to be satisfied for all time. Thus the cancellation condition is certainly not satisfied by all states that are reached by the end of the association phase.

The following section considers a more sophisticated approach, and obtains a stronger result.

7 An argument on the sign of coefficients in the cancellation condition

Let us return to the cancellation condition being satisfied for isolated state values.

We may discount the solution $\xi_2 = \xi_3 = 0$ as this only occurs in the trivial case of an association phase of zero duration.
Towards determining solutions of $d_2\xi_2 + d_3\xi_3 = 0$, or equivalently $\sum_{i=1}^{3} a_i e^{\lambda_i \cdot t} = 0$ $(0 = \lambda_1 > \lambda_2 > \lambda_3)$ let us attempt to establish the sign the constant coefficient (a_1) of the expression, given by **(E6.1)**.

Clearly $-k_a{}^*\alpha_1{}^*\beta_1$ on the denominator of **(E6.1)** is < 0. Consider the remaining numerator factor of currently unknown sign:

```
> unknown_sign_numer_zero_coeff:=collect_radicals(numer
  (extended_cc_const_coeff1)/(-beta[1]*alpha[1]*k[a]),
  [y2_descrim] ,sortlist);
```

$$unknown\_sign\_numer\_zero\_coeff := \left(k_{-2} + k_2\right)\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} + \left(k_{-2} - k_2\right)k_d - k_{-2}^2 - 2\,k_2\,k_{-2} - k_2^2 \quad \textbf{(E7.1)}$$

Let us determine where **(E7.1)** is zero:

```
> solve(unknown_sign_numer_zero_coeff=0,theta);
```

$$\left[\left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = 0,\ k_2 = k_2,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = k_d,\ k_2 = 0,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = 0\right]\right] \quad \textbf{(E7.2)}$$

Each of these solutions are infeasible. Thus, **(E7.1)** is not zero within the feasible

parameter space.

Evaluating **(E7.1)** for a randomly chosen feasible point of the parameter space

```
> Digits:=70; subs({ k[d]=3.0*10^(-3),k[2]=3.7*10^4,k[-2]=0.083},
  unknown_sign_numer_zero_coeff);
```

$$Digits := 70$$

$$4.037819394805341973216664775871195714196936985085 1\, 10^{-11} \qquad \textbf{(E7.3)}$$

As **(E7.1)** is continuous and non-zero on the feasible parameter space, the result **(E7.3)** suggests that it is positive everywhere.

Let's check the behaviour of **(E7.1)** with a computational approach.
Note that **(E7.1)** is defined for all positive values of the parameters as the radical is always non-negative by **(E5.1.3)**.

```
> Minimize(unknown_sign_numer_zero_coeff, assume=nonnegative);
```

$$[0., [k_{-2} \qquad \textbf{(E7.4)}$$

$$= 0.86848856906148039525069509648682615467831982782456495\backslash$$

$$0713167547627948,\ k_2$$

$$= 1.08141819218546911281345793390982060060437608662306953\backslash$$

$$6988843845586 8,\ k_d = 0.]]$$

Numerical results suggest that **(E7.1)** is positive for feasible (that is, positive) parameter values.

Let us show this analytically.

```
> diff_vec:=simplify(Gradient(unknown_sign_numer_zero_coeff,[k
  [d],k[-2],k[2]]));
```

$$diff\_vec := \Big(\Big(k_{-2} \sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2}$$

$$- k_2 \sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2} - k_{-2}^2 + k_d k_{-2} + k_2^2$$

$$+ k_d k_2 \Big) \Big/ \sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2} \Big) \bar{e}_{k[d]}$$

$$- \Big(2\,k_{-2} \sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2}$$

$$+ 2\,k_2 \sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2}$$

$$- k_d \sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2} - 2\,k_{-2}^2 - 4\,k_2 k_{-2}$$

$$+ 3\,k_d k_{-2} - 2\,k_2^2 - k_d k_2 - k_d^2 \Big) \Big/$$

$$\sqrt{k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 + 2\,k_2 k_{-2} + k_{-2}^2}\, \bar{e}_{k[-2]}$$

$$-\left(2\,k_{-2}\sqrt{k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2}\right.$$

$$+2\,k_2\sqrt{k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2}$$

$$+k_d\sqrt{k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2}-2\,k_{-2}^2-4\,k_2\,k_{-2}+k_d\,k_{-2}$$

$$\left.-2\,k_2^2-3\,k_d\,k_2-k_d^2\right)\Big/\sqrt{k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2}\,\bar{e}_{k[2]}$$

As the radical is always greater than zero for all positive parameter values, the derivatives are defined in the feasible region.

```
> zero_eval_cc_coeff_critical:=solve({diff_vec[1]=0, diff_vec[2]=
  0, diff_vec[3]=0},{k[d],k[-2],k[2]});
```

$$zero\_eval\_cc\_coeff\_critical := \{k_{-2}=k_{-2},\ k_2=k_2,\ k_d=0\} \qquad \textbf{(E7.5)}$$

This solution is not feasible as it is on the boundary of the parameter space where the gradient of the function does not exist.
As there are no critical points in the parameter space and **(E7.1)** is continuous and strictly positive in the parameter space, this suggests the function is positive everywhere.
Hence, a_1 given by **(E6.1)** is negative for all feasible parameter values.

The sign of a_2, the exp($\lambda_2 t$) coefficient

```
> extended_cc_coeff2:= collect(simplify(xi2_coeff*
  lambda2_coeff_vec[2,1]+ xi3_coeff*lambda2_coeff_vec[3,1]),
  sortlist):
```

To minimize unnecessary algebraic complexity, let us start by considering the denominator of this a_2 term:

```
> ext_cc_coeff2_denom:=factor(denom(extended_cc_coeff2));
```

$$ext\_cc\_coeff2\_denom := 2\left(\alpha_1\,k_a\,k_2+\alpha_1\,k_a\,k_{-2}+k_d\,k_{-2}\right)\left(\alpha_1\,k_a\right. \qquad \textbf{(E7.6)}$$

$$+\left(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}\right.$$

$$\left.\left.+k_2^2+2\,k_2\,k_{-2}+k_{-2}^2\right)^{1/2}-k_{-2}+k_2+k_d\right)$$

$$\left(\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2}+\right.$$

$$\left.k_{-2}^2\right)^{1/2}$$

As the radicand above can be written as

```
> expand((k[a]·alpha[1] + k[d] - k[2] -k[-2])^2 + 4·k[d]·k[2]);
```

$$\alpha_1^2\,k_a^2+2\,\alpha_1\,k_a\,k_d-2\,\alpha_1\,k_a\,k_2-2\,\alpha_1\,k_a\,k_{-2}+k_d^2+2\,k_d\,k_2-2\,k_d\,k_{-2}+k_2^2+2\,k_2\,k_{-2} \qquad \textbf{(E7.7)}$$

$$+k_{-2}^2$$

it is clearly non-negative in the feasible parameter space. Hence, all factors except one in **(E7.6)** are clearly non-negative. Let us consider the factor of indeterminate sign.

```
> qterm:= factors(ext_cc_coeff2_denom)[2][3][1];
```

$$qterm := \alpha_1^2 k_a^2 + 2\,\alpha_1 k_a k_d - 2\,\alpha_1 k_a k_2 - 2\,\alpha_1 k_a k_{-2} + k_d^2 + 2\,k_d k_2 - 2\,k_d k_{-2} + k_2^2 \quad \textbf{(E7.8)}$$
$$+ 2\,k_2 k_{-2} + k_{-2}^2$$

and determine where it is equal to zero:

```
> solve(qterm=0,theta);
```

$$\left[\left[\beta_1 = \beta_1,\ k_a = \frac{-k_d + k_2 + k_{-2} + 2\sqrt{-k_d k_2}}{\alpha_1},\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = k_{-2}\right],\left[\beta_1 = \beta_1,\right.\right. \quad \textbf{(E7.9)}$$
$$\left.\left. k_a = \frac{-k_d + k_2 + k_{-2} - 2\sqrt{-k_d k_2}}{\alpha_1},\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = k_{-2}\right]\right]$$

The first element of **(E7.9)** is infeasible as parameters are real and positive. The last element indicates that for one particular analyte concentration for a given pair of k_a and k_{-2} the term is zero.

This condition is certainly not satisfied for an entire experimental series, and is a set of measure zero in the parameter space.

To consider the sign of qterm, let us substitute into the expression a particular parameter value for which $k_{-2} > \alpha_1 * k_a$:

```
> subs({k[a]=0.0018,k[d]=0.0342,k[2]=700, k[-2]=0.0019,alpha[1]=
  1.0},qterm);
```

$$4.900480211628100\,10^5 \quad \textbf{(E7.10)}$$

and then substitute in a parameter value for which k[-2]< α_1*k[a] :

```
> subs({k[a]=0.0018,k[d]=0.0342,k[2]=700, k[-2]=0.0017,alpha[1]=
  1.0},qterm);
```

$$4.900477411764900\,10^5 \quad \textbf{(E7.11)}$$

As **(E7.8)** is continuous in the parameter space, **(E7.10)** and **(E7.11)** indicate that is positive everywhere in the parameter space except for where $k_{-2} = \alpha_1 * k_a$ where it is zero.

Thus, **(E7.8)** is always positive.

From this we note that **(E7.6)** (representing the denominator of a_2) is the product of three positive terms and hence is always positive.

Let us see if a further computational approach agrees. Minimizing the denominator of a_2

```
> Minimize(ext_cc_coeff2_denom,assume=nonnegative);
```

$$[0., [\alpha_1 \quad \textbf{(E7.12)}$$
$$= 0.14510011168546991570917952185446889913402236188696309 7\backslash$$

$$6981704689099294,\ k_{-2} = 0.,\ k_2 = 0.,\ k_a$$
$$= 0.14510011168546991570917952185446889913402236188696309 7\backslash$$
$$6981704689099381,\ k_d$$
$$= 0.02148714475147979932738632727203144070200462879999336 9\backslash$$
$$55422104140527320]]$$

yields an infeasible solution on the boundary of the parameter space. Hence, the denominator of a_2 is positive over the feasible parameter space. This does not contradict the results above.
Consider an analytical approach to finding critical points of qterm

```
> diff_vec2_denom:=simplify(Gradient(qterm,theta)):
> solve({diff_vec2_denom[1]=0,diff_vec2_denom[2]=0,
  diff_vec2_denom[3]=0,diff_vec2_denom[4]=0},theta);
```

$$[[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = 0,\ k_2 = 0,\ k_{-2} = \alpha_1 k_a]] \qquad \textbf{(E7.13)}$$

This solution is not a feasible critical point as the value given for k_2 is zero.

As the denominator of a_2 is positive almost everywhere, to determine the sign of the entire term we only need to consider
the behaviour of its numerator.

```
> ext_cc_coeff2_numer:=collect_radicals(numer
  (extended_cc_coeff2),[y1_descriminant, y2_descrim] ,sortlist);
```

$$ext\_cc\_coeff2\_numer := -2 k_d \beta_1 k_2 \left(\alpha_1^2 k_a^2 + 2 \alpha_1 k_a k_d - 2 \alpha_1 k_a k_2 - 2 \alpha_1 k_a k_{-2} \right. \qquad \textbf{(E7.14)}$$
$$\left. + k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2\right)^{3/2} + \left(\beta_1 k_2 \left(k_d^2 + 2 k_d k_2\right.\right.$$
$$\left.\left. - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2\right) + \left(-k_{-2} k_2 - k_2^2\right.\right.$$
$$\left.\left. + k_2 k_d\right) \beta_1 \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} - 2 \alpha_1 \beta_1 k_2 k_a k_d\right.$$
$$\left. + \left(-2 k_{-2} k_2 - 8 k_2^2\right) k_d \beta_1\right) \left(\alpha_1^2 k_a^2 + 2 \alpha_1 k_a k_d - 2 \alpha_1 k_a k_2 - 2 \alpha_1 k_a k_{-2} + k_d^2\right.$$
$$\left. + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2\right) + \left(\left(3 \alpha_1 \beta_1 k_2 k_a + 2 \beta_1 k_2 k_d\right) \left(k_d^2\right.\right.$$
$$\left.\left. + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2\right) + \left(k_{-2} k_2 + k_2^2\right.\right.$$
$$\left.\left. + k_2 k_d\right) k_a \beta_1 \alpha_1 \sqrt{k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + k_{-2}^2} + \left(\left(2 k_{-2} k_2\right.\right.\right.$$
$$\left.\left.\left. - 12 k_2^2\right) k_d - 4 k_2 k_{-2}^2 - 8 k_2^2 k_{-2} - 4 k_2^3\right) k_a \beta_1 \alpha_1\right)$$
$$\left(\alpha_1^2 k_a^2 + 2 \alpha_1 k_a k_d - 2 \alpha_1 k_a k_2 - 2 \alpha_1 k_a k_{-2} + k_d^2 + 2 k_d k_2 - 2 k_d k_{-2} + k_2^2 + 2 k_2 k_{-2} + \right.$$

$$k_{-2}^2\Big)^{1/2} - \beta_1\left(k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2\right)^2 k_2 + \Big(-k_a\,\beta_1\,k_2\,\alpha_1$$
$$+ \left(k_{-2}\,k_2 + k_2^2 - k_2\,k_d\right)\beta_1\Big)\left(k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2\right)^{3/2}$$
$$+ \Big(\left(5\,k_{-2}\,k_2 + 17\,k_2^2 - k_2\,k_d\right)k_a\,\beta_1\,\alpha_1 + \left(2\,k_{-2}\,k_2 + 8\,k_2^2\right)k_d\,\beta_1\Big)\left(k_d^2 + 2\,k_d\,k_2\right.$$
$$\left. - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2\right) + \Big(2\,k_{-2}\,k_2 + 8$$
$$k_2^2\Big)\,k_d\,k_a\,\beta_1\,\alpha_1\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} + \Big(\left(4\,k_{-2}^2\,k_2\right.$$
$$\left. + 4\,k_{-2}\,k_2^2 - 48\,k_2^3\right)k_d - 4\,k_{-2}^3\,k_2 - 24\,k_{-2}^2\,k_2^2 - 36\,k_{-2}\,k_2^3 - 16\,k_2^4\Big)\,k_a\,\beta_1\,\alpha_1$$

As $k_a\,\alpha_1\beta_1$ is clearly positive, let us restrict attention to just the factor of indeterminate sign:

```
> ext_cc_coeff2_numer_reduced:=simplify(numer(extended_cc_coeff2)
  /(beta[1]*alpha[1]*k[a])):
```

```
> solve(ext_cc_coeff2_numer_reduced=0,theta);
```

$$\Big[\left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = k_d,\ k_2 = 0,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = 0,\ k_2 = k_2,\ k_{-2}\right. \quad \textbf{(E7.15)}$$
$$\left. = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = \alpha_1\,k_a\right],\ \Big[\beta_1 = \beta_1,\ k_a = k_a,\ k_d =$$
$$\left. -\frac{\left(k_{-2} + k_2\right)k_a\,\alpha_1}{k_{-2}},\ k_2 = k_2,\ k_{-2} = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = 0,\ k_d = k_d,\ k_2 = k_2,\ k_{-2}\right.$$
$$\left. = k_{-2}\right],\ \left[\beta_1 = \beta_1,\ k_a = \frac{k_{-2}}{\alpha_1},\ k_d = k_d,\ k_2 = -k_d - k_{-2},\ k_{-2} = k_{-2}\right]\Big]$$

Of the possible results, the only feasible one is $\left[\beta_1 = \beta_1,\ k_a = k_a,\ k_d = k_d,\ k_2 = k_2,\ k_{-2} = \alpha_1 k_a\right]$.

Let us consider the behaviour of the function either side of $k_{-2} = \alpha_1 k_a$.

Consider when $k_{-2} > \alpha_1 k_a$:

```
> subs({k[a]=0.0018,k[d]=0.0342,k[2]=700, k[-2]=0.0019,alpha[1]=
  1.0,beta[1]=2.5},ext_cc_coeff2_numer);
```

```
-0.54298572750340968197729180507381275519031828155903164133\
                                                              7
                                  64
```
(E7.16)

and when $k_{-2} < \alpha_1 k_a$:

```
> subs({k[a]=0.0018,k[d]=0.0342,k[2]=700, k[-2]=0.0017,alpha[1]=
  1.0,beta[1]=2.5},ext_cc_coeff2_numer);
```

```
-0.54298572750340975992359217080119727192467785777549714250\
                                                              4
                                  99
```
(E7.17)

Consider a computational check on the sign of the numerator of a_2

```
> Digits:=150; f_max:=Maximize(ext_cc_coeff2_numer,assume=
  nonnegative): print(f_max):
```

$$Digits := 150$$

$$[0., [\alpha_1 = 0., \beta_1 = 0., k_{-2} \quad \textbf{(E7.18)}$$

$$= 2.546903033498443753427885148611444142260575861125290359\backslash$$

$$604639616037854849719756255666666622002988834774360503515\backslash$$

$$725997931474181160908346590539064899 20, k_2 = 0., k_a = 0., k_d = 0.]]$$

This result suggests that maximum of the function is very close to zero if not exactly zero.
Further, the maximum point found above is for an infeasible parameter value.
The numerical results are consistent with the result of the analytical approach that the function is not positive in the feasible parameter region.

By these results and continuity of the function **(E7.14)**, it is negative everywhere in the parameter space with the exception of the line $k_{-2} = \alpha_1 k_a$ where is it zero.
In this case, the coefficient of $\exp(\lambda_2 t)$ is of the indeterminate " 0/0 " form.
However, this occurs only for a set of measure zero in the parameter space.

Elsewhere in the parameter space, the numerator of a_2 **(E7.14)** is negative, its denominator **(E7.6)** is positive, and hence a_2 is negative overall.

We use this result in the classification of C in Chapter 5.

8 A result

Having demonstrated that pole-zero cancellation does not happen in general in $\mathcal{L}(y^{[2]})$, the form given by **(E4.2.3)** is the canonical form.
Hence, one may obtain the (useable) invariants from the denominator of the rational function and form the set of invariants

```
> phi[5]:=coeff(denom(LT_y2_simp),s); phi[6]:=coeff(denom
  (LT_y2_simp),s,0);
```

$$\phi_5 := k_{-2} + k_2 + k_d$$

$$\phi_6 := k_d k_{-2} \quad \textbf{(E8.1)}$$

```
> for i from 5 to 6 do: eq[i]:=phi[i]=subs({k[a]=Kappa[a],k[d]=
  Kappa[d],beta[1]=B[1],k[2]=Kappa[2],k[-2]=Kappa[-2]},phi[i]);
  od;
```

$$eq_5 := k_{-2} + k_2 + k_d = \mathrm{K}_{-2} + \mathrm{K}_2 + \mathrm{K}_d$$

$$eq_6 := k_d k_{-2} = \mathrm{K}_d \mathrm{K}_{-2} \quad \textbf{(E8.2)}$$

```
> IDequations:=[]; for i from 1 to 6 do; IDequations := [op
  (IDequations), eq[i]] end do:
```

$$IDequations := [\,] \qquad \textbf{(E8.3)}$$

```
> solset:=solve(IDequations,theta_prime);
```

$$solset := \left[\left[K_a = k_a,\ K_d = k_d,\ K_2 = k_2,\ K_{-2} = k_{-2},\ B_1 = \beta_1\right]\right] \qquad \textbf{(E8.4)}$$

This result shows that the structure is globally *a priori* identifiable.

9 An investigation of alternative plausible forms of $\mathcal{L}\left(y^{[2]}\right)$ in (E4.2.3) (an illustration of the SCUII algorithm)

The unprocessed form of $\mathcal{L}(y^{[2]})$ given in **(E4.2.3)** is a rational function of the form $\frac{a(s-z)}{(s-\lambda_s)(s-\lambda_l)}$. Given this form, without knowing whether or not the numerator and denominator are relatively prime, it is clear that the canonical form of the rational function is one of three possible types:

the unprocessed form given by **(E4.2.3)**,

a form of **(E4.2.3)** in which the factor $(s-\lambda_s)$ is cancelled to give an expression of the form $\frac{a}{(s-\lambda_l)}$,

a form of **(E4.2.3)** in which the factor $(s-\lambda_l)$ is cancelled to give an expression of the form $\frac{a}{(s-\lambda_s)}$.

The third form is not feasible by the earlier working, leaving only two possibilities for the canonical form of **(E4.2.3)**.

Suppose that each of the two remaining forms are treated in turn as if they are the canonical form of $\mathcal{L}(y^{[2]})$.
The a term in the numerator of each rational function depends on $x_{0,2}^{[2]}$ and $x_{0,3}^{[2]}$making it difficult to use, whereas the denominator coefficients are merely explicit functions of the rate constants.

By taking only these denominator coefficients from an assumed canonical form of $\mathcal{L}(y^{[2]})$ and adding them to $\phi^{[1]}$, (Equations **(E3.3.1)**-**(E3.3.4)**) a test of $\mathcal{C}$ for global *a priori* identifiability can proceed in each case. The worst result obtained from the two tests is the only judgement that can be accepted — the best result from the two tests may not be a correct judgement.

To show the value of the method, if both tests show that the system is at least

locally *a priori* identifiable then the system is clearly not unidentifiable. If both tests show that a system is globally *a priori* identifiable then there is no doubt that the system is in fact globally *a priori* identifiable.

Assume that $\mathcal{L}\left(y^{[2]}\right)$ given by (E4.2.3) is the canonical form and test $\mathcal{C}$ for global *a priori* identifiability.

This calculation was performed in **(E8.4)** and the structure was shown to be globally *a priori* identifiable.
Hence, let us consider the other candidates for the canonical form of L ($y^{[2]}$) that are alternatives to **(E4.2.3)**.

Assume cancellation of $\left(s - \lambda_s\right)$ occurs in (E4.2.3).

In this case only one invariant is available from the denominator of the assumed canonical form of $\mathcal{L}\left(y^{[2]}\right)$

```
> phi[5]:=lambda[l];
```

$$\phi_5 := -\frac{1}{2}\,k_{-2} - \frac{1}{2}\,k_2 - \frac{1}{2}\,k_d + \frac{1}{2}\,\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} \qquad \textbf{(E9.2.1)}$$

Setting up the equations for the identifiability test requires taking those from **(E3.3.6)** as these are from $\mathcal{C}^{[1]}$ and hence independent of the form of $\mathcal{L}\left(y^{[2]}\right)$, and adding to this list the equation obtained by using **(E9.2.1)**

```
> eqALT[5]:=phi[5]=subs({k[a]=Kappa[a],k[d]=Kappa[d],beta[1]=B
  [1],k[2]=Kappa[2],k[-2]=Kappa[-2]},phi[5]);
```

$$eqALT_5 := -\frac{1}{2}\,k_{-2} - \frac{1}{2}\,k_2 - \frac{1}{2}\,k_d + \frac{1}{2}\,\sqrt{k_d^2 + 2\,k_d\,k_2 - 2\,k_d\,k_{-2} + k_2^2 + 2\,k_2\,k_{-2} + k_{-2}^2} = -\frac{1}{2}\,K_{-2} - \frac{1}{2}\,K_2 - \frac{1}{2}\,K_d + \frac{1}{2}\,\sqrt{K_{-2}^2 + 2\,K_{-2}\,K_2 - 2\,K_{-2}\,K_d + K_2^2 + 2\,K_2\,K_d + K_d^2} \qquad \textbf{(E9.2.2)}$$

```
> IDequationsALT:=[eq[1],eq[2],eq[3],eq[4],eqALT[5]]:
```

and solving gives

```
> new_solset:=solve(IDequationsALT,theta_prime);
```

$$new\_solset := \left[\left[K_a = k_a,\ K_d = k_d,\ K_2 = k_2,\ K_{-2} = k_{-2},\ B_1 = \beta_1\right]\right] \qquad \textbf{(E9.2.3)}$$

Regardless of the value of the parameters there is a unique solution to the equations. If the form of $\mathcal{L}\left(y^{[2]}\right)$ assumed is the canonical form of $\mathcal{L}\left(y^{[2]}\right)$, then $\mathcal{C}$ is globally *a priori* identifiable.

Assume cancellation of

$(s - \lambda_1)$ occurs in (E4.2.3)

Cancellation of this factor was ruled out by **(E5.1.10)**, and hence there is no need to consider the form of L ($y^{[2]}$) that would result from cancellation of the factor.
However, it is considered here to illustrate the SCUII algorithm.

In this case (as for the previous case) only one invariant is available from the denominator of the assumed canonical form of L ($y^{[2]}$):

```
> phi[5]:=lambda[s];
```

$$\phi_5 := -\frac{1}{2}k_{-2} - \frac{1}{2}k_2 - \frac{1}{2}k_d - \frac{1}{2}\sqrt{k_d^2 + 2k_dk_2 - 2k_dk_{-2} + k_2^2 + 2k_2k_{-2} + k_{-2}^2} \quad \textbf{(E9.3.1)}$$

An equation is formed from this invariant and added to the four obtained from from $\mathcal{C}^{[1]}$

```
> eqALT[5]:=phi[5]=subs({k[a]=Kappa[a],k[d]=Kappa[d],beta[1]=B
  [1],k[2]=Kappa[2],k[-2]=Kappa[-2]},phi[5]);
```

$$eqALT_5 := -\frac{1}{2}k_{-2} - \frac{1}{2}k_2 - \frac{1}{2}k_d - \frac{1}{2}\sqrt{k_d^2 + 2k_dk_2 - 2k_dk_{-2} + k_2^2 + 2k_2k_{-2} + k_{-2}^2} = -\frac{1}{2}K_{-2} - \frac{1}{2}K_2 - \frac{1}{2}K_d - \frac{1}{2}\sqrt{K_{-2}^2 + 2K_{-2}K_2 - 2K_{-2}K_d + K_2^2 + 2K_2K_d + K_d^2} \quad \textbf{(E9.3.2)}$$

```
> IDequationsALT:=[eq[1],eq[2],eq[3],eq[4],eqALT[5]]:
```

and solving gives

```
> new_solset:=solve(IDequationsALT,theta_prime);
```

$$new\_solset := [[K_a = k_a, K_d = k_d, K_2 = k_2, K_{-2} = k_{-2}, B_1 = \beta_1]] \quad \textbf{(E9.3.3)}$$

which shows that the assumed canonical form of L ($y^{[2]}$) leads to a classification of C as globally *a priori* identifiable.

A conclusion

Considering the result of **(E9.3.3)** alongside those of **(E9.2.3)** and **(E8.4)** shows that regardless of the true canonical form of **(E4.2.3)**, using any of the three possible forms of L ($y^{[2]}$) leads to the conclusion that C is globally *a priori* identifiable. As a result, it is certain that $\mathcal{C}$ is globally *a priori* identifiable. This shows that it is not necessary to know the canonical form of L ($y^{[2]}$) in order to classify C.

Hence, in the case of the model structure under consideration, the SCUII algorithm gives a result which is both definite and conclusive.

Appendix F

Maple code and results in support of Conjecture 5.1

Numerical results in Support of Conjecture 5.1

The program below uses a computational method to determine the solutions to the cancellation condition corresponding to λ_s.

```
> restart( ) : with(LinearAlgebra) : with(VectorCalculus) : with(plots) :
    with(Statistics) :
```

```
> kernelopts(maxdigits); kernelopts(version);
```

38654705646

Maple 2015.2, X86 64 LINUX, Dec 20 2015, Build ID 1097895 **(F1)**

Defining the structure for the two state conformational change model

Let us develop the DE system describing the association phase of a BIAcore experiment.

Starting by defining the state vector

```
> X:= PositionVector( [x[1](t),x[2](t),x[3](t)], cartesian[x,y,z]
  ) ;
```

$$X := \begin{bmatrix} x_1(t) \\ x_2(t) \\ x_3(t) \end{bmatrix} \quad \textbf{(F1.1)}$$

and its time derivative

```
> Xdot := diff(X, t);
```

$$Xdot := \begin{bmatrix} \frac{d}{dt} x_1(t) \\ \frac{d}{dt} x_2(t) \\ \frac{d}{dt} x_3(t) \end{bmatrix} \quad \textbf{(F1.2)}$$

and the system matrix that defines the dynamics of the interactions

```
> A1:=Matrix(3,3,[-k[a]*alpha,k[d],0, k[a]*alpha, -(k[d] + k[2]),
  k[-2], 0, k[2], -k[-2] ]);
```

$$A1 := \begin{bmatrix} -k_a \alpha & k_d & 0 \\ k_a \alpha & -k_d - k_2 & k_{-2} \\ 0 & k_2 & -k_{-2} \end{bmatrix} \quad \textbf{(F1.3)}$$

which allows us to form the right-hand side of the first-order ODEs describing the rates of change of concentrations of interacting species

```
> eqn_rhs := MatrixMatrixMultiply(A1,X);
```

$$eqn\_rhs := \begin{bmatrix} -k_a \alpha x_1(t) + k_d x_2(t) \\ k_a \alpha x_1(t) + (-k_d - k_2)\, x_2(t) + k_{-2} x_3(t) \\ k_2 x_2(t) - k_{-2} x_3(t) \end{bmatrix} \quad \textbf{(F1.4)}$$

```
> eq:=[]: for i from 1 to 3 do: eq:=[op(eq), Xdot[i] = eqn_rhs[i]
  ]: end do: eq;
```

$$\left[\frac{d}{dt} x_1(t) = -k_a \alpha x_1(t) + k_d x_2(t),\ \frac{d}{dt} x_2(t) = k_a \alpha x_1(t) + (-k_d - k_2)\, x_2(t) + k_{-2} x_3(t),\ \frac{d}{dt} x_3(t) = k_2 x_2(t) - k_{-2} x_3(t)\right] \quad \textbf{(F1.5)}$$

These rates are subject to a conservation of mass condition

```
> eq := [op(eq), x[1](t) + x[2](t) + x[3](t) = beta[1]];
```

$$eq := \left[\frac{d}{dt} x_1(t) = -k_a \alpha x_1(t) + k_d x_2(t),\ \frac{d}{dt} x_2(t) = k_a \alpha x_1(t) + (-k_d - k_2)\, x_2(t) + k_{-2} x_3(t),\ \frac{d}{dt} x_3(t) = k_2 x_2(t) - k_{-2} x_3(t),\ x_1(t) + x_2(t) + x_3(t) = \beta_1\right] \quad \textbf{(F1.6)}$$

Let us solve for the states, subject to the initial conditions

```
> ics := x[1](0) = beta[1], x[2](0) = 0, x[3](0) = 0;
```

$$ics := x_1(0) = \beta_1,\ x_2(0) = 0,\ x_3(0) = 0 \quad \textbf{(F1.7)}$$

```
> Xsol := dsolve({eq[1], eq[2], eq[3], eq[4], ics}, [X[1], X[2], X[3]]) :
```

A numerical exercise to determine the solutions to the cancellation condition xi[3] = *K**xi[2] for a range of parameter values

Recall the *K* term from the cancellation condition:

```
> K := -( -k~_2 - k~_-2 + k~_d
      + sqrt(k~_d^2 + 2 k~_d k~_2 - 2 k~_d k~_-2 + k~_2^2 + 2 k~_2 k~_-2 + k~_-2^2) ) / ( -k~_2
      - k~_-2 - k~_d
      + sqrt(k~_d^2 + 2 k~_d k~_2 - 2 k~_d k~_-2 + k~_2^2 + 2 k~_2 k~_-2 + k~_-2^2) ) :
```

In the exercise to follow, the solutions of the cancellation condition will be determined for a variety of parameter values.
Define uniform random variables to pseudo-randomly generate feasible values of the parameters.

```
> upper := [1.0·10^4, 1, 1.0·10^4, 1, 100]; U := Vector[row](5); for i from 1 to 5 do;
      U[i] := RandomVariable('Uniform'(0, upper[i])) : od;
```

$$upper := [10000.0,\ 1,\ 10000.0,\ 1,\ 100]$$

$$U := 0e_{x1}$$

$$U_1 := \_R$$
$$U_2 := \_R0$$
$$U_3 := \_R1$$
$$U_4 := \_R2$$
$$U_5 := \_R3 \qquad \textbf{(F2.1)}$$

The parameters are collected in the vector

```
> theta := [k[a], k[d], k[2], k[-2], beta[1]];
```

$$\theta := [k_a, k_d, k_2, k_{-2}, \beta_1] \qquad \textbf{(F2.2)}$$

A routine for numerically determining the times at which the cancellation condition is satisfied

This routine returns the N·3 matrix of results : the run number in col 1, result(s) from the root finding in col 2, the number of solutions in col 3

```
> cancellation_condition_solns:= proc(N,theta,acc,Xsol,K,
  results)  ::  real;
  local i, j, l, thetaval, termx2, termx3, Kterm, multCount,
  temp, fail, MinVal, MaxVal, failPercentage, cond ;
  global U;
> Digits := acc;  #digits used in the computations
>  thetaval := Vector(nops(theta));
  for i from 1 to N do:
        for j from 1 to nops(theta) do: thetaval[j] := Sample(U[j], 1)[1];
    end do:
    thetaval := convert(thetaval, list) : cond := [alpha = 0.01] :
       for l from 1 to 5 do: cond := [op(cond), theta[l] = thetaval[l]] : od:
    termx3 := rhs(subs(cond, Xsol[3])) : termx2 := rhs(subs(cond, Xsol[2])) :
    Kterm := subs(cond, K) :
    results[i, 1] := i;  results[i, 2] := fsolve( termx3 - Kterm·termx2 = 0, t,
    -1.0..1000, real);
    temp := results[i, 2] :
  if (whattype(temp) = function) then temp := [ ]: else temp := [temp]: end
    if: results[i, 3] := numelems(temp) :
  end do:
> multCount := 0 : for i from 1 to N do; if results[i, 3] > 1 then multCount
      := multCount + 1; end if; od;
> print("multiple solutions count", multCount)
      # zero shows that there is not any cases of two or more  solutions.
>  end proc;
```

```
cancellation_condition_solns := proc(N, θ, acc, Xsol, K, results)::real;
  local i, j, l, thetaval, termx2, termx3, Kterm, multCount, temp, fail,
  MinVal, MaxVal, failPercentage, cond;
  global U;
  Digits := acc;
```

(F2.1.1)

```
    thetaval := VectorCalculus:-Vector( nops(θ) );
    for i to N do
        for j to nops(θ) do
            thetaval[j] := Statistics:-Sample(U[j], 1)[1]
        end do;
        thetaval := convert(thetaval, list);
        cond := [α = 0.01];
        for l to 5 do cond := [op(cond), θ[l] = thetaval[l]] end do;
        termx3 := rhs(subs(cond, Xsol[3]));
        termx2 := rhs(subs(cond, Xsol[2]));
        Kterm := subs(cond, K);
        results[i, 1] := i;
        results[i, 2] := fsolve(termx3 + VectorCalculus:- `-`(Kterm
        * termx2) = 0, t, -1.0..1000, real);
        temp := results[i, 2];
        if whattype(temp) = function then
            temp := [ ]
        else
            temp := [temp]
        end if;
        results[i, 3] := numelems(temp)
    end do;
    multCount := 0;
    for i to N do
        if 1 < results[i, 3] then multCount := multCount + 1 end if
    end do;
    print("multiple solutions count", multCount)
end proc
```

A routine to processes the output in preparation for plotting results

```
> results_processing:=proc(N, results, GraphMat) :: real;
    local i, fail;
    GraphMat := results;
> fail := 0:
    for i from 1 to N do;
    if (whattype(GraphMat[i, 2]) = function) then GraphMat[i, 2] := undefined;
        fail := fail + 1;
    end if;
```

```
    end do;
> print("no. failures to solve equation=", fail, "failure percentage=",
      evalf[3]((fail / N) * 100)) :
    end proc;

results_processing := proc(N, results, GraphMat)::real;                    (F3.1)
    local i, fail;
    GraphMat := results;
    fail := 0;
    for i to N do
        if whattype(GraphMat[i, 2]) = function then
            GraphMat[i, 2] := undefined; fail := fail + 1
        end if
    end do;
    print("no. failures to solve equation=", fail, "failure percentage=", evalf
    [3](100*fail*1 / N))
end proc
```

For the first run, the root finding procedure is called with inputs

```
> N := 10^5; acc:= 30; seed:=1; randomize(seed);
  #set the seed of the pseudo-random number generator for
  reproducibility of the runs to follow.
                                  N := 100000
                                  acc := 30
                                  seed := 1
                                     1                                      (F3.2)
```

and the results matrix is initialized

```
> results := Matrix(N, 3):
> ts := time( ) :    # time of process commencement
> cancellation_condition_solns(N,theta,acc,Xsol,K,results) ;
  duration:= time() - ts;
                        "multiple solutions count", 0
                            duration := 1118.917                            (F3.3)
```

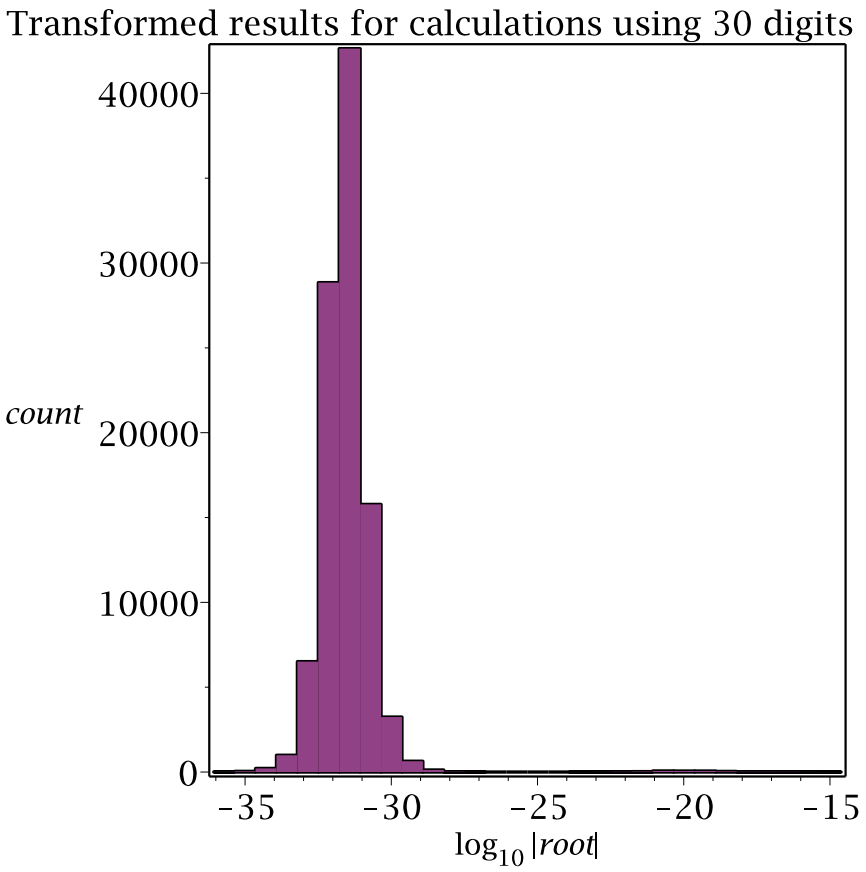
Call the results processing routine to clean up the output to remove cases where no solution was found:

```
> results_processing(N,results,GraphMat);
      "no. failures to solve equation=", 654, "failure percentage=", 0.654  (F3.4)
```

To show the distribution of roots, the following histogram shows the log in base 10 of the absolute value of roots returned.

```
> Histogram( map(log10, map(abs, Column(results, 2)) ), frequencyscale
```

= *absolute*, *ignore* = *true*, *color* = "Niagara DarkOrchid", *labels*
= [$\log_{10}$abs(*root*), *count*], *title*
= "Transformed results for calculations using 30 digits");



This particular simulation shows that for the 10^5 runs, whenever a solution is returned for *t*, its absolute value is rarely greater than 10^{-30}, and hence is quite close to zero. This is as expected by the conjecture.
To ascertain if these roots are non-zero as a consequence of the precision of the calculations, the exercise is repeated using more significant digits.
The state of the pseudo-number generator is set as a result of the last uniform random variate generated in the previous run.

```
> unassign('results'); unassign('GraphMat'); results := Matrix(N, 3) : N := 10^5;
    acc := 70;
```

$$N := 100000$$
$$acc := 70 \qquad \textbf{(F3.5)}$$

```
> ts := time( ) :
> cancellation_condition_solns(N,theta,acc,Xsol,K,results) ;
  duration:= time() - ts;
```

"multiple solutions count", 0

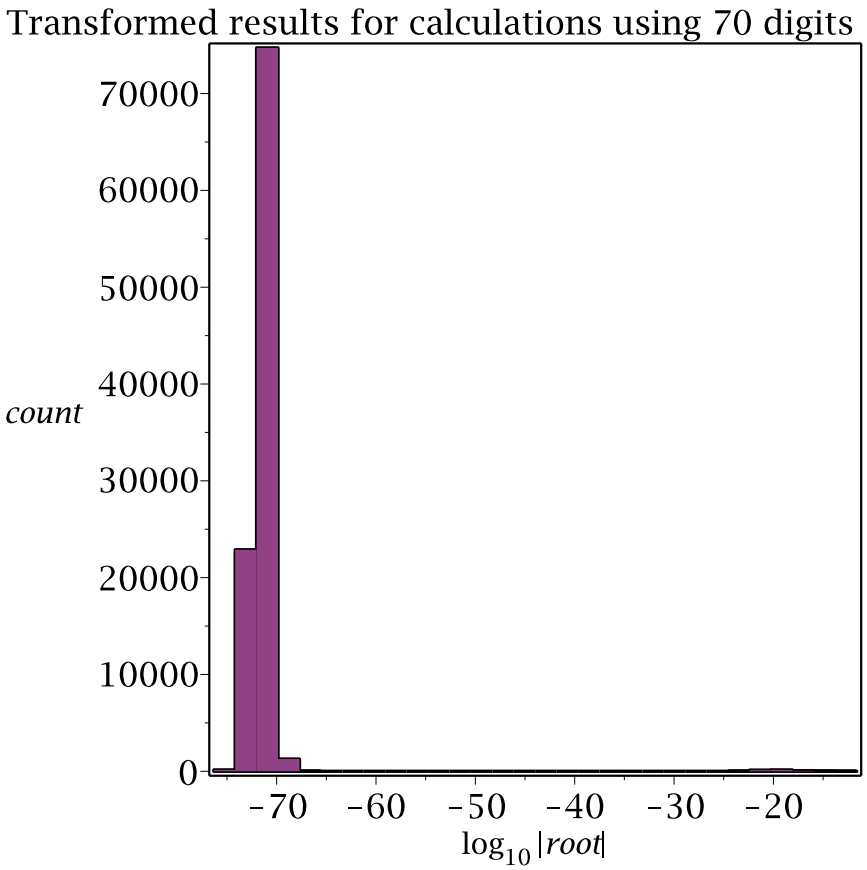
$$duration := 1282.958 \quad \textbf{(F3.6)}$$

```
> results_processing(N,results,GraphMat);
```

"no. failures to solve equation=", 645, "failure percentage=", 0.645 **(F3.7)**

Display the results of the root-finding exercise

```
> Histogram( map(log10, map(abs, Column(results, 2)) ), frequencyscale
    = absolute, ignore = true, color = "Niagara DarkOrchid", labels
    = [log10abs(root), count], title
    = "Transformed results for calculations using 70 digits");
```



The results above show that when 70 digits are used in the root finding calculation, the absolute values of the results rarely exceed $t = 10^{-70}$.
This shows that in each case (each set of parameters used in the exercise) the time at which the cancellation condition is satisfied is very close to zero.
Further, the results are substantially closer than the results obtained when 30 significant digits are used in the calculations.
The closeness of these results to zero supports the conjecture.

Appendix G

A Maple routine for simplification of algebraic expressions featuring radicals

Elements of the literature on simplification of symbolic expressions are summarised in Bailey *et al.* [4]. Many of the references included are concerned with a particular type of expression. Bailey *et al.* [4] add to this literature by proposing a set of algorithms called "SimplifySum", intended for the simplification of expressions of the form $\sum_i \alpha_i x_i$ where each α_i is rational and each x_i is real or complex. Their suite of algorithms is implemented in Mathematica. Their approaches are quite general, however, they are not intended for parametric expressions. While it may be possible to adapt the algorithms for this purpose and then implement them in Maple, they seem unnecessarily complex for our specific task of collecting radicals.

Hence, we propose a relatively simple Maple routine that is adequate for our purposes. Consideration of linear switching system structure test cases with more than three states is likely to produce more complex expressions than those seen for the two-state conformational change model. These test cases may encourage further investigation of the methods presented in Bailey *et al.* [4].

A programme able to simplify expressions involving radicals

Application of the SCReMI algorithm in testing the `two-state conformational change model' for global *a priori* identifiability in Chapter 6 is an algebraically complex exercise.
The algebraic expressions obtained tend to involve radicals.
Experimentation with combinations of native Maple commands has found that they are not able to collect radicals in complex expressions. This makes certain expressions unnecessarily complicated.
Resolving this matter will aid the simplification of expressions.
The routine presented below takes a simplistic yet effective approach to the problem which is sufficient for the purposes of this thesis.
Given an input containing multiple radical expressions, by replacing each by a simple symbol, the routine is able to collect like terms using standard Maple commands.

```
> kernelopts(version);
```

Maple 2015.2, X86 64 LINUX, Dec 20 2015, Build ID 1097895 **(G1)**

To illustrate the performance of native Maple commands in sorting multivariate expressions, consider a simple expression:

```
> simple:= k[-2] + k[2]^2 + k[d] + k[a]*alpha[1];
```

$$simple := \alpha_1\, k_a + k_2^2 + k_{-2} + k_d \qquad \textbf{(G2)}$$

and an expression involving two distinct radicals:

```
> original:=k[a]*sqrt(k[2]+k[-2]+k[d])+k[-2]*sqrt(k[a]*alpha[1]+k
  [d])+k[a]+ k[-2]*sqrt(k[a]*alpha[1]+k[d])+k[2]*k[-2]*sqrt(k[a]*
  alpha[1]+k[d])
  + k[d]*sqrt(k[2]+k[-2]+k[d]);
```

$$original := k_a\sqrt{k_2 + k_{-2} + k_d} + 2\,k_{-2}\sqrt{\alpha_1\, k_a + k_d} + k_a + k_2\, k_{-2}\sqrt{\alpha_1\, k_a + k_d} + k_d\sqrt{k_2 + k_{-2} + k_d} \qquad \textbf{(G3)}$$

Expressions **(G2)** and **(G3)** are similar to those seen in the two-state conformational change model considered in Chapter 6.
This list dictates the preferred sorting of symbols in the expression:

```
> sortlist := [k[a], alpha[1], k[d], k[2], k[-2]];
```

$$sortlist := [k_a, \alpha_1, k_d, k_2, k_{-2}] \qquad \textbf{(G4)}$$

Application of standard sorting commands gives the desired sorting for the simple expression:

```
> sort(simple,sortlist);
```

$$k_a\,\alpha_1 + k_2^2 + k_d + k_{-2} \qquad \textbf{(G5)}$$

However, Maple commands applied to the more complex expression:

```
> collect(sort(original,sortlist),sortlist);
```

$$\left(\sqrt{k_d + k_2 + k_{-2}} + 1\right) k_a + \sqrt{k_a\,\alpha_1 + k_d}\; k_2\, k_{-2} + 2\sqrt{k_a\,\alpha_1 + k_d}\; k_{-2} + \sqrt{k_d + k_2 + k_{-2}}\; k_d \qquad \textbf{(G6)}$$

are unable to collect like terms.

One approach to the problem is given by the following programme:

```
> collect_radicals:=proc(expression,radicand_list,sortlist)
  # Maple 16 is not readily able to group radicals together in
  order to simplify expressions.
  # This proceedure takes a radicand, makes a substitution to
  enable a simplification, then
  # replaces the original expression.
  local i, Z, eqn_sub_set, reverse_reln, new_sortlist, new_exp,
  new_exp_final;
  # add the substitution variable to the start of the list of
  parameters for the sorting operation.
  Z:=convert(Vector(nops(radicand_list),symbol=Z),list); print("Z",
  Z);
  new_sortlist:=[op(Z),op(sortlist)]; print("new_sortlist",
  new_sortlist);
  # Replace the radical term i with Z[i]^2 such that the square
  root term is simplified such that usual collection
  # routines work to sort and collect terms.
  eqn_sub_set:=[]; for i from 1 to nops(radicand_list) do;
  eqn_sub_set:=[op(eqn_sub_set),radicand_list[i]=Z[i]^2]; od;
  print("eqn sub set", eqn_sub_set);
  new_exp:=simplify(simplify(expression,eqn_sub_set),symbolic);
  print("new exp v1", new_exp);
  # simplify the expression
  new_exp:=collect(sort(simplify(new_exp,radical, symbolic),
  new_sortlist),new_sortlist); print("new exp v2", new_exp);
  # replace the artificial variable with the radicand in terms of
  the original parameters.
  reverse_reln:=[];
  for i from 1 to nops(radicand_list) do; reverse_reln:=[op
  (reverse_reln),Z[i]=sqrt(radicand_list[i])]; od; print("reverse
  the original substitution", reverse_reln);
  new_exp_final:=new_exp;
  for i from 1 to nops(radicand_list) do; new_exp_final:=subs
  (reverse_reln[i],new_exp_final); od;
  # this was ok, but didn't pick up all powers of Z1, so use
  algsubs as more general, but can only apply one condition at a
  time     #new_exp_final:=subs(reverse_reln,new_exp);
  return(new_exp_final);
> end proc;
```

```
collect_radicals := proc(expression, radicand_list, sortlist)                    (G7)
    local i, Z, eqn_sub_set, reverse_reln, new_sortlist, new_exp, new_exp_final;
    Z := convert(Vector(nops(radicand_list), symbol = Z), list);
    print("Z", Z);
    new_sortlist := [op(Z), op(sortlist)];
```

```
    print("new_sortlist", new_sortlist);
    eqn_sub_set:= [ ];
    for i to nops(radicand_list) do
        eqn_sub_set:= [op(eqn_sub_set), radicand_list[i] = Z[i]^2]
    end do;
    print("eqn sub set", eqn_sub_set);
    new_exp:= simplify(simplify(expression, eqn_sub_set), symbolic);
    print("new exp v1", new_exp);
    new_exp:= collect(sort(simplify(new_exp, radical, symbolic), new_sortlist),
    new_sortlist);
    print("new exp v2", new_exp);
    reverse_reln:= [ ];
    for i to nops(radicand_list) do
        reverse_reln:= [op(reverse_reln), Z[i] = sqrt(radicand_list[i])]
    end do;
    print("reverse the original substitution", reverse_reln);
    new_exp_final:= new_exp;
    for i to nops(radicand_list) do
        new_exp_final:= subs(reverse_reln[i], new_exp_final)
    end do;
    return new_exp_final
end proc
```

Define the list of radicands to be substituted for in the original expression:

```
> radicand_list:=[k[a]*alpha[1]+k[d], k[2]+k[-2]+k[d]]; nops
  (radicand_list);
```

$$radicand\_list := \left[k_a\,\alpha_1 + k_d,\ k_d + k_2 + k_{-2}\right]$$

$$2 \qquad \textbf{(G8)}$$

and applying the routine to the "original" expression:

```
> output := collect_radicals(original, radicand_list, sortlist);
```

$$\text{"Z"},\ \left[Z_1,\ Z_2\right]$$

$$\text{"new\_sortlist"},\ \left[Z_1,\ Z_2,\ k_a,\ \alpha_1,\ k_d,\ k_2,\ k_{-2}\right]$$

$$\text{"eqn sub set"},\ \left[k_a\,\alpha_1 + k_d = Z_1^2,\ k_d + k_2 + k_{-2} = Z_2^2\right]$$

$$\text{"new exp v1"},\ Z_1\,Z_2^2\,k_{-2} - Z_1\,k_{-2}^2 - Z_1\,k_{-2}\,k_d + 2\,Z_1\,k_{-2} + Z_2\,k_a + Z_2\,k_d + k_a$$

$$\text{"new exp v2"},\ \left(Z_2^2\,k_{-2} - k_{-2}^2 - k_{-2}\,k_d + 2\,k_{-2}\right)Z_1 + \left(k_a + k_d\right)Z_2 + k_a$$

$$\text{"reverse the original substitution"},\ \left[Z_1 = \sqrt{k_a\,\alpha_1 + k_d},\ Z_2 = \sqrt{k_d + k_2 + k_{-2}}\right]$$

$$output := \left(\left(k_d + k_2 + k_{-2}\right)k_{-2} - k_{-2}^2 - k_{-2}\,k_d + 2\,k_{-2}\right)\sqrt{k_a\,\alpha_1 + k_d} + \left(k_a\right. \qquad \textbf{(G9)}$$

$$+ k_d)\sqrt{k_d + k_2 + k_{-2}} + k_a$$

The expression given by **(G9)** shows that the routine has obtained a simplified form of the original expression

```
> original;
```

$$\sqrt{k_a \alpha_1 + k_d}\, k_2 k_{-2} + \sqrt{k_d + k_2 + k_{-2}}\, k_a + k_a + \sqrt{k_d + k_2 + k_{-2}}\, k_d + 2\sqrt{k_a \alpha_1 + k_d}\, k_{-2} \qquad \textbf{(G10)}$$

Export to an archive so I can call it from other Maple files, wouldn't it be nice IF IT WORKED:

```
> savelib('collect_radicals', "CollectRadicals.mla") :
>
```